Atmospheric pressure plasma jets: characterisation and interaction with human cells and bacteria

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Atmospheric Pressure Plasma Jets: Characterisation and Interaction with Human Cells and Bacteria

PROEFSCHRIFT

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Summary

Atmospheric Pressure Plasma Jets: Characterisation and Interaction with Human Cells and Bacteria

Cold atmospheric pressure plasma jets (CAPPJs) are highly reactive, strongly non-equilibrium plasmas which operate close to room temperature and are able to treat heat sensitive surfaces. The goal of this thesis is to gain deeper insights into the characteristics of CAPPJs and their interaction with eukaryotic and prokaryotic cells.

In the last decade, cold atmospheric pressure plasmas have been shown to be a promising tool for e.g. sterilization (e.g MRSA) and wound healing (such as enhancing the healing process of diabetic foot ulcers). In spite of these many promising results, many open questions remain, concerning the mechanisms of the interaction of plasmas with biological matter.

CAPPJs exist in various different configurations, including different electrode configurations, gas(-mixtures), driving frequencies and voltage waveforms. Due to the large variety of CAPPJs used in the plasma community, a direct comparison of the various plasma sources is an extremely difficult task and slows down the progress in the understanding of the mechanisms of the plasma interaction with biological cells and its dependence of different plasma parameters.

This work focuses on the characterization of a single plasma jet source and on the investigation of the interaction between this plasma source and prokaryotic and eukaryotic cells by performing in vitro measurements of various parameters. The plasma jet is operational in a large parameter space, which includes a cross and a linear electric field electrode configuration, the excitation includes mono-polar nanosecond voltage pulses, continuous and kHz modulated radio frequency (RF) voltages. Helium and argon have been used as the carrier gas with admixtures of molecular gases up to a few percent.

In order to investigate the plasma, several diagnostic methods have been applied in the course of this work. For the RF plasma jet a power dissipation measurement method is developed in order to measure and control the power dissipated in the...
plasma.

Measurements of the gas temperature inside the plasma have been obtained with the rotational emission spectrum of OH(A-X) and N\(_2\)(C-B) for an argon and a helium plasma, respectively. It is shown that the rotational temperature of those molecules is in agreement with the gas temperature, as validated by Rayleigh scattering.

Measurements of the gas temperature of the plasma effluent have been obtained with a thermocouple and showed that the temperature gradient between the plasma and the plasma effluent of argon is much steeper compared to a helium plasma of the same length, for the investigated plasma jet. This is the reason why, for the RF plasma, argon was used as main carrier gas in this work.

The RF plasma has been modulated with a kHz modulation to reduce the gas temperature, while maintaining a high plasma power. With the modulation of the voltage signal it was possible to link the power dissipation to the morphology of the plasma during an RF cycle. It was shown that when the plasma jet is in direct contact with a conductive surface, the emission of the plasma as well as the plasma power is increasing. This effect was more pronounced for a linear electric field electrode configuration compared to a cross electric field configuration. To avoid these complex effects, a remote treatment was performed in this work with the plasma jet in cross field configuration.

Since in most medical applications, a liquid layer, such as blood, water, etc. is present, treatments of prokaryotic and eukaryotic cells have been performed in hundreds of microlitres of solutions, including distilled water, saline solutions and (nutritionally rich) buffered solutions, such as lysogeny broth (LB) or phosphate buffered saline (PBS). Treating the bacteria in the various media with the kHz modulated RF argon plasma jet, it is shown that the highest inactivation of bacteria (\textit{Pseudomonas aeruginosa}) is reached with an unbuffered medium due to, among other things, the reduction of the pH during plasma treatment.

To get a better insight of the bacteria inactivation mechanism, the various species of the so called "plasma cocktail" have been measured and evaluated. Diagnostics included absolute UV measurements and absolute density measurements of the reactive species in the effluent of a plasma by molecular beam mass spectrometry (MBMS). In case of a remote plasma treatment, nitric oxide, ozone and hydroxyl, play the main role in the plasma induced liquid phase chemistry which leads to bactericidal effects. With a 0-dimensional solution kinetics model it was shown that the influx of those reactive species reduces the pH and creates species in concentrations high enough to be bactericidal (such as HNO\(_2\) and ONOO\(^-\)) or species at concentrations which inhibit bacteria growth (such as H\(_2\)O\(_2\)).

Next to \textit{P. aeruginosa}, eukaryotic cells, such as fibroblasts and keratinocytes have been treated with the RF plasma to evaluate the selectivity of the RF plasma jet between eukaryotic and prokaryotic cells. It is shown that if the treatment and incubation time is short enough, \textit{P. aeruginosa} can be effectively inactivated,
while the cell viability is not significantly affected. To investigate if the plasma has any effect on cell proliferation the mono-polar nanosecond pulsed plasma has been used CMT-93 cells (a mouse cell-line) together with a real time cell analyser. No statistical significant plasma induced cell proliferation has been observed for the investigated treatment conditions.

In addition, more fundamental studies have been performed for the mono-polar nano second pulsed plasma which is often termed as "plasma bullet" or "guided streamers". The similarity between the plasma bullets in an argon-air interface, which are stable in time and space and randomly created streamers in pure gases was investigated by creating guided streamers of argon in an argon atmosphere. It is showed that a high repetition frequency and a forced gas flow are the main criteria for a guided streamer. Due to the stability of a guided streamer, time and spatial resolved electron densities of the guided streamer in an argon atmosphere have been obtained, for the first time, with Thomson scattering.

The results of this work provided a deeper insight into the parameters and species of cold atmospheric pressure plasma jets as well as their interaction with cells sus-
pended in different types of liquid media. A better understanding of these plasma sources will help to evaluate and optimize their use for biomedical applications such as wound healing and sterilisation.
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Chapter 1

Introduction*

1.1 Non-equilibrium plasmas

Plasmas are (partly) ionised gases and consist of photons, neutral, reactive and charged species and are the main contributors of visible matter in the universe and therefore of various songs about the night sky\(^1\). “Man-made” plasmas play also an important role in many technologies and everyday life, most prominently present as light sources.

Plasmas can be divided in either thermal or non-thermal plasmas. A non-thermal plasma is, opposite to a thermal plasma (e.g. the sun, fusion reactors, etc.), not in thermal equilibrium, which means it cannot be described by a single temperature. In most cases, the heavy neutrals and ions have a lower mean energy, i.e. lower temperature, than the high energetic electrons. This feature enables effective reactive chemistry in the gas phase driven by electrons with a temperature of a few electron volts (eV), while the overall gas temperature remains orders of magnitude lower, even up to ambient temperature, making non-thermal plasmas an ideal source for surface treatments of heat sensitive material, such as polymers, textiles and even biological tissues. Some well known applications of non-thermal plasmas in surface treatment processes can be found for example in etching or surface modification processes (see e.g. [1] and references therein).

Man-made plasmas are in most cases created by applying a voltage across two electrodes in a background gas. If this voltage exceeds a certain voltage (i.e. the

\(^{*}\)Parts of the introduction are taken and modified from the author's publications listed in the subsequent chapters.

\(^{1}\)e.g. “The sun is a miasma of incandescent plasma.” - from the song “Why Does the Sun Really Shine” from the band “They Might be Giants”.
breakdown voltage), some molecules and atoms in the gas will ionise and create electron avalanches, which lead to the formation of a stable plasma discharge. This breakdown voltage depends on different factors. For two parallel plate electrodes and a DC-voltage the breakdown voltage depends for example on the type of gas, pressure and inter-electrode distance. The breakdown voltage is described by the Paschen law [2].

The Paschen law basically states, that the applied voltage needs to be high enough, to accelerate electrons fast enough to ionise enough atoms/molecules in the gas. If the pressure is too low or the distance between the electrodes is too small, the electrons will not be able to collide and ionise with enough atoms or reach a high enough energy to ionise, respectively. Similarly, for a too large distance of the electrodes or a too high pressure the voltage needs to increase. This implies that in between these extremes of $p \cdot d$ a minimum of the breakdown voltage can be reached. As the voltage to sustain a plasma is typically smaller than the breakdown voltage, a large breakdown voltage which is sustained after breakdown leads to rapid ionisation and heating.

The existence of this minimum in $p \cdot d$ is the reason why many non-thermal plasma applications are realised in low pressure systems in reactors of a few tens of cm size. In addition, gas heating, which proceeds through electron-molecules collisions, is slow at low pressure and plasmas therefore can typically operate at low gas temperatures.

### 1.2 Cold atmospheric pressure plasmas jets

If the plasma needs to be created at atmospheric pressure due to reasons such as in-line processing (as opposed to batch processes at low pressure) or treatment of living cells, a special electrode or reactor design is necessary.

At atmospheric pressure the plasma is prone to arcing. An arc is a thermal plasma that forms when a current is high enough to heat up (one of) the electrodes. If the temperature at the electrode is high enough more electrons are created due to thermionic emission, which leads to a very high current at low voltage at high gas temperatures. Arcs are very energetic and need to be avoided in many applications. The most prominent sources which create non-arcing atmospheric pressure plasmas are dielectric barrier discharges (DBDs), corona discharges and cold atmospheric pressure plasma jets (CAPPJs). The latter are the focus of this work.

Many different designs of atmospheric pressure plasma jets exist [3–5]. The characteristic feature of all of them is that usually a rare gas, such as helium or argon, is blown through a $\mu$m or mm size nozzle. In between or surrounding this nozzle are in most cases one electrode which is connected to a high (0.1 to 100 kV) voltage source and a grounded electrode. The plasma is then usually created inside
the nozzle between these electrodes and blown out into the open air.

To prevent arcing in plasma jets three methods can be applied either separately or in various combinations. One method is to use a DBD geometry, with the two electrodes being separated by at least one dielectric barrier, such as glass. The advantage of the dielectric barrier is, that charge, which is created in the volume of the plasma, will deposit on the dielectric. This leads to a local reduction of the electric field, which will basically lead to a self quenching of the discharge.

Another method is to apply a voltage with a high frequency, usually RF \[6\] in order to reduce the operation voltage. In most cases these RF plasmas are capacitively coupled plasmas, i.e. created between two electrodes at radio frequency, e.g. 13.56 MHz. With this configuration the current is mainly displacement current, compared to the usually much smaller ohmic current at these frequencies. The last method is to apply the voltage and switch it off before a transition to a spark/arc can occur, i.e. applying voltages with a duration of pulses of a few hundreds of nanoseconds or less, depending on the electrode geometry.

There are several advantages of plasma jets over DBD discharges. DBD discharges, especially in air, are in most cases filamentary and are produced randomly over the surface, while a plasma jet forms in most cases a stable diffuse looking discharge without the need of the surface as a secondary electrode. This makes it possible to treat rough surfaces homogeneously in either a direct contact or an indirect mode, which is a challenge in a DBD geometry. The common disadvantages of plasma jets are the relatively small size of the discharges and the need of (expensive) rare gases. Although air jets have also been developed \[7\].

As mentioned before, many different variations of electrode configurations and voltage waveforms can and are used in different plasma research groups. Since all of these plasma sources are different in many ways [size, gas(-flow), electrode], a direct comparison of the different plasma sources is in most cases extremely challenging if not impossible without a lot of detailed information of plasma properties.

### 1.2.1 The plasma source

One of the goals of this thesis was to use a single plasma source, which was able to operate in various discharge modes in order to directly compare the different characteristics. The plasma source we decided on consists of a tungsten needle on which the voltage is applied. It is surrounded by a quartz glass tube. A gas flow of usually 1 to 2 slm of helium or argon, sometimes with admixtures of molecular gases, is applied through the glass tube to force the gas and the plasma into the open air. Due to the high electric field on the needle tip the plasma can be operated either with an argon or a helium flow.

Two different types grounded electrodes have been used in this work and are illustrated in figure 1.1, i.e. a plate electrode with a 5 mm hole which was placed
in front of the glass tube and a ring electrode which fitted around the glass tube. These geometries were used in order to investigate either a linear field (an electric field linear to the gas flow), or a cross-field configuration (an electric field perpendicular to the gas flow), respectively [8].

In addition, two different excitation voltages have been further investigated: radio frequency (13.56 MHz), in continuous mode and modulated at 20 kHz, and 500 ns mono-polar voltage pulse with a repetition rate between 1 and 5 kHz. In the following sections the main characteristics of these sources are listed as well as the research questions we have addressed in this work.

1.2.2 RF jets

Radio frequency plasmas are used because the breakdown voltage for these sources is relatively low [3, 6] and the plasmas can be operated at relative low gas temperatures. Different research groups have investigated plasma jets driven in the MHz range. In recent years various diagnostic methods have been applied on these jets, ranging from gas temperature measurements by optical emission spectroscopy (OES), to measurements of the amount of reactive or metastable species [9–11] with laser, emission/absorption spectroscopy or mass spectrometry.

Since the electrons follow the high frequency field, while the ions are static the amount of electron-atom/molecule collision is higher than for jets with lower driving frequencies. This makes these type of discharges a rich source of reactive species. Several diagnostic methods have been applied to characterize the RF jet used in this work in terms of reactive species. van Gessel et al. measured densities of nitric oxide in the plasma with air admixtures with LIF of the order of $10^{21}$ m$^{-3}$ [12]. Zhang et al. measured ozone densities of the order of $10^{21}$ m$^{-3}$ with UV absorption spectroscopy [13] and atomic oxygen densities of the order
of $10^{22} \text{m}^{-3}$ with TALIF [14]. Verreycken et al. has measured for a similar RF jet absolute OH densities up to $10^{21} \text{m}^{-3}$ in the case of an Ar-H$_2$O mixture feed gas [15].

Electron densities of an argon plasma in the range of $10^{19} \text{m}^{-3}$ to $10^{20} \text{m}^{-3}$ and electron temperatures in the range of 1 eV to 1.5 eV have been obtained by these jets by van Gessel et al. with Thomson scattering [16]. In terms of dynamics it has been shown by the mentioned co-workers that the electron temperature is modulated by the electric field strength, while the electron density remains constant during the RF cycle. The same is valid for concentration of the reactive species, which even for a kHz modulation are not time modulated.

The main disadvantages of RF plasmas are that the plasma length and temperature both scale with the plasma dissipated power, which makes it challenging to create cold (around room temperature) RF plasma jets. The most common ways to reduce the temperature is either by, limiting the plasma to low powers [17], using a high gas flow [18], or by applying a time modulation of the plasma with a small enough duty cycle [19]. The latter approach is also used in this work.

One important aspect in these devices is the controllability and comparability. For high power RF plasmas (tens of watts to kW) or low pressure RF plasmas (which usually have high reactance), the power delivered to the plasma is a convenient parameter to measure and use as a control parameter. However, the measurement of the dissipated power of small size cold atmospheric pressure RF plasma jets is a non trivial task. In most of these cases the reactance is of the order of magnitude of the electrical voltage probes. Probes connected directly to the driving electrode will change the coupling significantly and introduce systematic errors in the voltage and therefore power measurements. To compensate this effect in high power plasmas, the power of the plasma and the matching network are then often measured by assuming that the plasma power is much higher than the power dissipated inside the matching network. However, as for cold atmospheric pressure RF plasmas, the power is very low and of the order of magnitude of the power of the matching network, this method is also not directly applicable.

Although a lot of diagnostic methods have been applied to investigate RF plasma jets, some questions remain open, which will be addressed in this thesis:

- Is there an easy method to accurately measure the plasma dissipated power of small, low power (kHz modulated) RF plasma jets?

- Are there reliable optical emission spectroscopy techniques to obtain accurately the gas temperature and/or the electron density? In what parameter range can they be used?

- What are the reactive species and ion densities/fluxes of a RF jet delivered to a defined substrate?
• How accurately can reactive species be measured with a mass spectrometer operated at atmospheric pressure?

• What is the effect of the substrate properties on the plasma properties of a RF plasma jet?

1.2.3 Nanosecond pulsed kHz jets

To obtain plasmas at room temperature with a relatively low gas flow many research groups use pulsed voltages with a pulse width of a few hundreds of nanoseconds, and a repetition rate of the order of 1 kHz. In these cases, when operated in air at atmospheric pressure, so-called plasma bullets can be observed by taking nanosecond time resolved images of the plasma [20, 21].

A plasma bullet is basically the visible ionisation front of a propagating streamer [22] and is perhaps better called “guided streamer”. This term was introduced by Boeuf et al. and indeed is much more consistent with the occurring physics as a streamer is not merely a plasma package or bullet but is connected by a plasma channel to the pin electrode [23].

A streamer is a self propagating ionisation front. Characteristic for a streamer is its tip, called “streamer head”, which consists of a high space charge density creating a high electric field exceeding the applied electric field, which allows the streamer head to propagate even into low electric field regions. The Raether-Meek criterion [24, 25] states that the creation of a streamer depends on $\alpha d$, i.e. the Townsend coefficient and the length of the electron avalanche. This transition is often limited by the electrode gap distance, which has to reach a critical value of 20 to form a streamer head with a localised electric field high enough to create and sustain the self-propagation.

Dielectric barrier discharges at atmospheric pressure are one example where streamers are created and result in a filamentary discharge. However for some applications it is often desirable to obtain diffuse Townsend or glow discharges instead of filamentary discharges. Basically the objective is to prevent streamer formation and ensuring that the Raether-Meek criterion is not satisfied. This can be achieved by slowing down the ionisation rate by decreasing the voltage, using different gas mixtures mixtures for which the ionisation is a two-step process such as Penning ionisation or increasing the pre-ionisation [26, 27].

Depending on the polarisation of the voltage pulse applied to the electrode a negative or a positive streamer can be created. If a gas mixture contains species emitting photons with a higher energy than the ionisation energy of other species in the gas (e.g. O$_2$-N$_2$-mixtures) photoionisation is an important ionisation process for creating the electrons in front of the streamer head, especially for positive streamers.

Another source of electrons is background ionisation. The origin of the charged
species can be, e.g. cosmic radiation, radioactivity or charged particles and meta-
stable atoms which remained from a previous discharge. The last mechanism is
often termed “memory effect” and is observed at discharges with a high repetition
frequency, since in these circumstances a significant amount of metastables and/or
charged species is still present when the next high voltage pulse starts [25]. It has
been observed that this effect can decrease the breakdown voltage [28, 29] or helps
microdischarges sustain the same patterns in dielectric barrier discharges [30].

One difference between the "guided" streamers in the plasma jets and the "nor-
mal" streamer discharges in uniform gases is the reproducibility of the guided
streamer in space and time while most of the typical corona discharges tend to
branch randomly [31]. The reason for branching is still under debate. Presently
Laplacian instability of the ionisation front [32, 33] or inhomogeneities in the
gas [34, 35] are used to explain branching. The first one, also called "Laplacian
growth" handles the streamer branching as a branching phenomena observed usu-
ally at plane interfaces during mixing processes of one medium into another under
the influence of a Laplacian field. Small deviations of the propagating plane front
spontaneously lead to branching [36]. The other approach is to assume randomly
placed seed electrons or any other randomly distributed source or sink of charge
existent at the path of propagation, which can spatially change the ionisation
rate.

Research presented in [31, 37] has shown that the number of branches increases
by an increase of the voltage, i.e. the electric field and energy. In the case of
microwave plasmas the number of filamentation/branches depends on the po-
wer/energy dissipation in the discharge which has been clearly observed at atmo-
spheric pressure by Cardoso et al. [38]. Although the latter is at rather different
conditions, both cases can somehow be linked to the Raether-Meek criterion, since
energy dissipation is directly linked to the electron density and the critical electron
density determines the field of the streamer head and thus the $\alpha$-coefficient.

It is clear that for a guided streamer one needs a guidance (i.e. favourable direction
of the streamer) and high enough ionisation rates to fulfil the Meek-criterion
although the energy dissipation has a limit as otherwise branching will occur. For
plasma bullets created in open air, the guidance is sustained by the gas channel
created by the flow of the jet in an air surrounding, due to the higher effective
first Townsend coefficient in the channel [23, 39].

Plasma bullets in open air are reported by various research groups [20, 21, 40–45],
but almost no work has been done by comparing these bullets with streamer
discharges in pure argon or helium atmospheres [37, 46, 47]. Work on streamers
in pure atmospheres usually focusses on point-like electrode geometries inside a
gas without a directed gas flow surrounding the needle (see [22] and references
therein), usually excited by high voltage pulses (tens of kV) and at low repetition
frequencies (below kHz).

Since understanding the plasma sources, used for the applications in this work, is
beneficial for understanding the mechanisms behind any application, the following questions concerning the ns-pulsed kHz plasma jets are addressed in this thesis:

- Is it possible to create plasma bullets in pure atmospheres, i.e. control the branching of streamers, where the guidance due to a air-rare gas channel is not present?
- What is the electron density and temperature which drive the chemistry of a (guided) streamer (time and spatially resolved)?
- What are the reactive species densities compared to an RF jet?

### 1.3 Plasmas interacting with cells and tissues

As mentioned above cold atmospheric pressure plasmas can be used to treat heat sensitive material such as living cells. However, the question rises what benefit of treating tissues and cells with a plasma could exist.

The reason why plasmas might be a promising tool for medical treatments lies in the various components the plasma can produce, including UV photons, electric fields, ionic and reactive species. All of these components are already used separately for many years in medicine for different applications, however, in a plasma a large range of synergistic mechanisms can be achieved, which, if the plasma is cold enough (i.e. not much higher than room temperature) and electrically safe it could be used in wound healing applications, such as in treatments for burn wounds.

Already in 1945 Alexander Fleming, who won the Nobel prize for the discovery of the bactericidal effects of penicillin warned in his speech that bacteria can become resistant to antibiotics\(^1\). Bacteria resistant to antibiotics are becoming an increasing problem in the last years in hospitals and are according to Grundmann et al. a global threat [49]. Cosgrove et al. estimated that antimicrobial drug-resistant infections increase death, illness and costs by 30 to 100 % [50].

Next to drug resistance, antibiotics also have side effects for some patients. As shown by Greenberger et al. 10 % of patients are allergic against penicillin, although, however, only 10 % of those patients show allergic reactions during antibiotic treatment [51]. Wistrom et al. showed that diarrhoea has been observed in 5 % during antibiotic treatment [52]. These issues show the need of new ways for treating bacterial infections.

In this context cold atmospheric pressure plasmas could be a novel way for effective bacteria treatment. In 1988 Sato et al. showed for the first time antimicrobial

\(^1\)Quote: "Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant" [48]
effects obtained with a cold atmospheric pressure plasma [53]. Zimmermann et al. estimated for their plasma source and treatment conditions that the secondary resistance has been estimated to have a lower limit of $10^{-30}$, i.e. 1 bacterium out of $10^{15}$ kg bacterial biomass could become resistant to a plasma treatment, which is very improbable [54]. No primary resistance against the plasma treatment has been detected, but a statistical lower limit of $10^{-10}$ is given. This value depends on the method which was used to obtain the primary and secondary resistance.

Bacterial infections are a common problem with burn wound patients. As patients with severe burn wounds have a compromised immune system, they are much more susceptible to infections of opportunistic bacteria such as *Pseudomonas aeruginosa*. This kind of infections can spread easily via, for example, contact with medical personnel or improperly designed sinks [55]. Avoiding infections in burn wounds leads to lower mortality (up to 28 %) [56].

Although plasmas are already used in applications such as electrosurgery [57, 58] and a great amount of research efforts has been put into decontamination of medical instruments [59], using cold atmospheric pressure plasmas for wound healing is a relative new research topic and has still many open questions, particularly about long term safety, amongst other things.

In the past years many different research groups used different plasma sources with various bacteria and showed the antimicrobial effects of those plasma sources (see e.g. [60] and references therein). It has been seen that usually for a remote plasma treatment of bacteria and cells, the main component responsible for the bactericidal effects are long living reactive species such as NO and ozone, [61–66] in some cases also with contributions of (V)UV-photons [67, 68]. If a direct treatment is used, additional factors such as ionic species and short lived reactive species come in to play [69, 70].

Different cold atmospheric pressure plasmas have been studied intensively for several decades to inactivate bacteria in liquids [71]. In general, bacteria have been tested on agar plates [62, 72], in distilled water [73, 74] or (buffered) saline solution [75] while eukaryotic cells have typically been tested in culture media such as DMEM [76]. The different media are important because it has been shown that plasma inactivation of bacteria can strongly depend on the solution in which the bacteria are treated [73, 77, 78]. The presence of buffers in culture media will e.g. reduce the effect of plasma treatments on bacteria. However, a systematic study of the effects of plasma treatment on both prokaryotes and eukaryotes under the same conditions has not been performed. The search for a realistic model, which has relevance for *in vivo* experiments, is far from trivial. There are only few reports on treatment of bacteria and eukaryotic cells. An infected wound model based on the scratch assay was used to study plasma treatments [79], but bacterial inactivation was not quantified or studied in detail. Non-thermal argon plasma was found to be bactericidal for the intracellular bacterial pathogen *Chlamydia trachomatis*, while immediate mortality of the fibroblasts was not apparent but not investigated further [80].
The large variety of plasma properties available for cold atmospheric plasmas often makes it challenging to find an optimum between inactivating the bacteria and maintaining the wound healing potential. There is still debate on the active components in the plasma induced liquid chemistry, which act on the cells. Due to the many different plasma sources, which are used all over the world, it is possible that the discrepancies found in different labs are related to differences in chemical composition of the plasma, the plasma induced liquid chemistry and the target cells. Recent work favours NO related chemistry as key component for the inactivation of bacteria for some plasma sources [62, 78, 81]. Reactive Oxygen Species (ROS), more specifically O$_2$ - related chemistry [82, 83] or O$_3$ [75] have also been reported to play a role for specific conditions. Recent results on eukaryotic cells show that both Reactive Nitrogen Species (RNS) and ROS are involved in cell response [66, 76, 84].

The goal of this work within the frame work of plasma interactions with cells can be summarised as follows:

- What role does a liquid surrounding the bacteria/cells play in plasma-cell interaction?
- What is the most important plasma parameter and the mechanism in bacteria inactivation?
- Are there plasma conditions for which mammalian cells in solution survive while bacteria in the same solution are efficiently inactivated?
- Is there an operation of the plasma jet where treated mammalian cells show enhanced proliferation?

1.4 Thesis outline

To summarize, the following topics corresponding to the research goals presented above will be addressed in this thesis. Chapter 2 to 6 deal with the RF jet, while chapter 7 to 9 deal with the ns-pulsed kHz jet.

- Chapter 2 introduces the plasma source used with MHz frequency (RF) and the power measurements. Gas temperature and electron densities have been obtained to characterize the plasma jet operated in helium and argon to find the relevant conditions for biomedical treatments.
- Chapter 3 introduces the RF plasma jet operated in kHz modulation mode and investigates the argon plasma jet for two different electrode configurations in contact with different substrates. This chapter motivates why most bio treatments we performed were obtained under remote conditions and gives a perspective for treatments when the jet is in contact with the sample.
Chapter 4 introduces the mass spectrometer measurements and characterizes the plasma in terms of reactive and ionic species fluxes produced by the RF plasma jet. The air entrainment is also discussed together with the decay of the ion density as a function of distance in the far effluent of the jet.

In chapter 5 the plasma jet is used for bacterial inactivation of *P. aeruginosa* in distilled water and the main components of the plasma resulting in the observed bacteria inactivation are presented. The results include UV, gas temperature, mass spectrometry, ion chromatography and colorimetric measurements which are combined and used to validate a simple but order of magnitude accurate liquid chemistry model. The model enables to quantify the important species created inside the liquid medium, which are responsible for bacteria inactivation.

Chapter 6 focuses on the selectivity of the plasma jet, reporting for the first time treatments of a prokaryotic cell (*P. aeruginosa*) and eukaryotic cells (fibroblasts and keratinocytes) in the same liquid medium, i.e. saline solution. Treatment conditions which inactivate bacteria but keep the cell activity of eukaryotic cells unaffected are presented.

In chapter 7, a guided streamer, stable in space and time in a uniform gas is reported and the similarities between this discharge and the plasma bullets in open air are experimentally investigated. It is shown that not only the gas channel but also pre-ionisation can be used to form guided streamers.

In chapter 8 for the first time electron densities and electron temperatures are presented for a guided argon streamer discharge in pure argon and in ambient air with Thomson scattering.

The effect of the ns-pulsed kHz jet operated in helium on prokaryotic and eukaryotic cells is investigated. Its selectivity in terms of bacteria killing (*P. aeruginosa*) and the ability of inducing cell proliferation of eukaryotic cells (CMT 93) is reported in chapter 9.

Chapter 10 summarizes the key findings of this thesis.
References


Chapter 2

Power dissipation, gas temperatures and electron densities of helium and argon RF CAPPJs *

Abstract

A set of diagnostic methods to obtain the plasma parameters including power dissipation, gas temperature and electron density is evaluated for an atmospheric pressure helium or argon RF plasma needle for biomedical applications operated in open air. Different methods are investigated and evaluated to obtain the gas temperature. In this chapter the gas temperatures obtained by rotational spectra of OH(A-X), \( \text{N}_2 \)\(^2\) (B-X) and \( \text{N}_2 \)\(^2\) (C-B) are compared with Rayleigh scattering measurements and measurements of the line broadening of hydrogen and helium emission lines. The obtained gas temperature ranges from 300 to 650 K, depending on the gas. The electron densities are estimated from the Stark broadening of the hydrogen \( \alpha \) and \( \beta \) lines which yield values between \( 10^{19} \) and \( 10^{20} \) m\(^{-3} \). In the case of helium, this is an overestimate as is shown by a power balance from the measured power density in the plasma jet. The obtained plasma parameters enable to explain the radial contraction of the argon plasma compared to the more diffuse helium plasma. The accuracy of all considered diagnostics is discussed in detail.

*This chapter is a modified version of the publication: S. Hofmann, A.F.H. van Gessel, T. Verreycken, P. Bruggeman - Power dissipation, gas temperatures and electron densities of cold atmospheric pressure helium and argon RF plasma jets, Plasma Sources Sci. Technol. 20 (2011) 065010 (12pp)
2.1 Introduction

To get a better insight of the plasma-bio interaction, characterisation of the plasma source in terms of gas temperature, electron density ($n_e$) and absorbed power is an important but non-trivial task. Non-trivial as the gas temperature has to be below 42°C to prevent cell destruction [1] and very accurate gas temperature measurements are thus required. A similar challenge is present for the power dissipation as at low power dissipation in the plasma, the power dissipation in the matchbox becomes non-negligible and needs to be taken into account. At small electron densities line broadening measurements are complex because other broadening mechanisms than Stark broadening become dominant.

The power dissipation of the plasma is a basic physical property of the plasma which can almost directly lead to estimates of $n_e$. One method to obtain the power of RF plasmas is to use commercial thermal power meters. However, in this method power dissipation of the matchbox is an important factor as it can be bigger than the power dissipation in the plasma. Additionally these methods are often not applicable for time dependent power measurements, e.g. for time modulated RF driven plasmas [2].

Voltage and current probes can be directly connected to the electrodes, allowing to calculate the power dissipation of the plasma [3, 4]. In the case of atmospheric pressure plasma jets (APPJJs) however the capacity of the probes is in the same order of magnitude as the capacity of the source. Due to this and the large resistance of the source, the introduction of the probe influences the impedance of the circuit significantly [5].

To measure the gas temperature optical emission spectroscopy is an often used non-invasive method [6, 7]. The rotational spectrum of molecules like OH, $N_2$, $N^+_2$ is widely used for temperature determination. However, in this case the rotational states should follow a Boltzmann distribution and should be in equilibrium with the gas temperature. This is for atmospheric pressure plasmas in most conditions the case but not in general as Bruggeman et al. have shown with atmospheric pressure plasmas in and near liquids [8].

Another method for gas temperature determination is Rayleigh scattering of photons by atoms and molecules. In this method the intensity of the scattered laser light of the plasma is compared to a reference signal with a known gas temperature in the same gas composition to determine the gas temperature of the plasma [9, 10]. However, laser diagnostics are not always available and have to be used with care in order not to change the parameters of the plasma due to e.g. photo ionisation, especially for plasmas with low ionisation degree and large metastable densities, which is the case for APPJJs. Furthermore this method cannot be used for helium plasma jets with air around and inside of the plasma, since the Rayleigh scattering cross section of helium is small compared to air (see next sections).
The electron density can give information about the chemical reactivity of the plasma source. The broadening of atomic lines is a well known method to determine the electron density [6, 11]. In cases of high electron densities, large gas temperatures and low pressure, other line broadening effects such as the van der Waals broadening are negligible and mostly not taken into account. On the other end of the regime (low electron densities, low gas temperatures, atmospheric pressure) the van der Waals and Stark broadening are in the same order of magnitude and both have to be taken into account in the calculations. If one of the broadenings would not be considered, this would otherwise lead to significant overestimated electron densities or underestimated gas temperatures.

From the above it is obvious that, especially in the regimes of interest for biomedical applications, the mentioned diagnostic methods have to be used with care. This chapter focuses on a direct comparison of the gas temperature, electron density and power dissipation of an RF argon and helium plasma needle. We are comparing the different diagnostics itself as well as the results obtained for the different gases and for different current root mean squares ($I_{\text{rms}}$) to get a detailed insight into the physical parameters of the APPJs.

This chapter starts with a description of the experimental setup (section 2.2) and the details of the used instruments for the measurements. The next section (section 2.3) deals with the theoretical aspects and diagnostics we used to obtain the plasma parameters. In section 2.4 the results of power measurements, gas temperature and electron densities are presented. The second to last section (section 2.5), preceding the conclusion (section 2.6), contains the discussion of the results and an evaluation of the accuracies of the presented methods for APPJs. The morphology of the discharge is also discussed.

### 2.2 Experimental setup

Figure 2.1 shows a schematic of the experimental setup. The plasma source consists of an RF driven tungsten needle with a diameter of 1 mm and a sharpened tip surrounded by a glass tube with a 3 mm outer and a 1.5 mm inner diameter. A gas flow of argon, helium or a mixture of 10% argon in helium, referred to as "helium-argon-mix" in the following sections, is applied through the tube. The gas flow is kept constant at 1 standard litre per minute (SLM). The discharge is thus ignited in these gas compositions. The setup is not in a vacuum vessel and thus open to air. The RF frequency is 11.7 MHz generated by a signal generator (Agilent, 20 MHz Function Generator) connected to a power amplifier (Amplifier Research 75 W, 5-250 MHz). A conductance (coil) is connected in series between the high voltage output of the power amplifier and the plasma source in order to match the amplifier to the plasma source.

A grounded copper electrode is positioned at a distance of 5 mm from the needle. It has a hole with a diameter of 5 mm through which the plasma can penetrate.
Figure 2.1: Experimental Setup.

This electrode simulates a two electrode system used in other research groups with a concentric electrode around the tube [4, 12, 13], while allowing access for laser spectroscopy of the active region between the two electrodes. The plasma jet is a so called linear field plasma jet, since the electrical field and the gas flow have the same direction [14].

Voltage and current probes (Tektronix - P5100, Pearson Current Monitor - 2877) are connected between the power amplifier and the matching network to an oscilloscope (Agilent Technologies, 350 MHz, 2 GSa/s). Optical emission spectroscopy and Rayleigh scattering is used for the determination of the gas temperature of the active zone of the plasma. For Rayleigh scattering a YAG laser (second harmonics, 532 nm) is used and focused at around 2 mm after the glass tube to prevent back scattering of the laser on the glass or secondary electrode. More details on the Rayleigh measurement and the used laser setup can be found in [15, 16].

For the line broadening and the measurements of the rotational spectra an optical fiber is used which collects the emission from the same position at which the Rayleigh measurements are performed. For the line broadening this fiber is connected to a Double Echelle monochromator with an Andor-CCD Camera which has a spectral resolution between 6 to 8 pm between 480 to 660 nm. For the rotational spectrum a Jobin Yvon monochromator (HR 1000 M) backed with a SBIG-CCD camera is used with a spectral resolution of around 22 pm which allows a broader wavelength range for single shot measurements of molecular bands.
2.3 Theoretical aspects and methods

2.3.1 Dissipated power and power density

The average dissipated power $P$ during one period $T = \frac{1}{f}$ can be calculated with

$$P = \frac{1}{T} \int_{0}^{T} U(t) \cdot I(t) dt,$$  \hspace{1cm} (2.1)

with $U(t)$ and $I(t)$ being the measured voltage and current waveforms. The disadvantage of this method is that a voltage probe introduces another capacity into the circuit. This changes the impedance of the setup and therefore strongly influences the power coupling to the plasma.

To reduce these errors the voltage and current probes are connected between the power amplifier and the matching network. The drawback of this method is, that the power dissipation of the matching network and the plasma is measured, rather than the power dissipation of the plasma alone.

We measured the temperature (and thus the heat dissipation in the resistance) of the coil with and without a gas flow, i.e. with and without a plasma. These measurements confirmed that the same heat dissipates in the coil with and without the plasma for the same current. This allows to measure the power dissipation in the plasma as follows:

$$P_{\text{diss}}(I_{\text{rms}}) = P_{\text{on}}(I_{\text{rms}}) - P_{\text{off}}(I_{\text{rms}}).$$  \hspace{1cm} (2.2)

with $I_{\text{rms}}$ being the root mean square current, $P_{\text{diss}}$, $P_{\text{on}}$ and $P_{\text{off}}$ being the dissipated power, the power with a gas flow and the power without a gas flow, respectively. The time delay induced by the current and voltage probe is corrected for by measuring with the probes the deviation of the 90° phase shift for a known vacuum capacitor.

Figure 2.2 shows the measured voltage and current with and without a plasma at the same amplified current. It can be seen that the phase shift between voltage and current is reduced while the plasma is on. The reason is the decreasing capacitive nature of the load and the increasing power dissipation in the plasma, leading to a more resistive load [4].

Figure 2.3 shows an example of the measured power as a function of $I_{\text{rms}}$ for a helium plasma. The power measured with and without a plasma is shown. Without a gas flow (and no plasma) the majority of the power dissipates in the resistive part of the coil. Fitting this power to a quadratic function reveals the $I^2$ dependency between power dissipation at the coil and the root mean square current as also seen by Benedikt et al. [17].
2.3.2 Optical diagnostics

Rotational temperature

Since the discharge operates in open air, impurities, like water, are always present in APPJs. The emission of the OH(A-X)-band is for several discharges one of the most intense emission and often used to determine the gas temperature of plasmas. With the relative intensities of the rotational lines of Q, P and R-branches of the rotational spectrum of OH(A-X)\( (0,0) \) the rotational temperature can be identified by simulating theoretical rotational spectra for different rotational temperatures (with spectra simulation programs such as Specair [18] or Lifbase [19]). The best fit between the experiment and the simulation reveals the rotational temperature of the experiment.

These programs, however, assume a Boltzmann distribution of the rotational levels in order to get a rotational temperature. This is for most atmospheric pressure plasma discharges a valid assumption, since the excited states have a large number of thermalizing collisions during their radiative life time, allowing the excited OH molecules to thermalize before emitting photons. Assuming that the rotational temperature is equal to the gas temperature, the gas temperature can be measured with this method.

However, previous work by Bruggeman et al. [8, 15, 20] has shown, that also in some cases for atmospheric pressure plasmas the rotational states do not follow a Boltzmann population distribution due to of different population mechanisms of the rotational states and collisional quenching which reduces the lifetime of the excited states at atmospheric pressure significantly. Note that in the case of Ver-
Figure 2.3: $P_{\text{off}}, P_{\text{on}}$ as a function of the $I_{\text{rms}}$ in the case of a helium plasma.

reycken et al. even when the rotational population distribution was a Boltzmann distribution it can lead to an overestimate of the gas temperature [15].

With a high resolution spectrometer the Boltzmann plot method can be used to determine the rotational temperature. Using the relative intensities of isolated lines of the rotational spectrum, $I_{\text{rel}}$, and

$$I_{\text{rel}} \propto A_{JJ'} (2J + 1) \lambda_{JJ'} \exp \left( - \frac{E_J}{kT_{\text{rot}}} \right),$$

(2.3)

with $A_{JJ'}$ as the Einstein coefficient taken from [21], $J$ and $J'$ as the rotational quantum number of the upper and the lower state, respectively, $E_J$ the energy of the upper level, taken from [22], $k$ the Boltzmann constant and $T_{\text{rot}}$ the rotational temperature, one can plot $\ln \left( \frac{I_{\text{rel}} \lambda_{JJ'}}{A_{JJ'} (2J + 1)} \right)$ as a function of the energy $E_J$. If the levels are Boltzmann distributed the slope yields the rotational temperature.

Figure 2.4 shows an example of the Boltzmann plot method for an argon and a helium case. As can be seen in the graph the values do follow a linear slope, indicating a Boltzmann distribution of the rotational states. Only isolated lines corresponding with an intensity larger than 5% of the maximum intensity are used.

For further comparison the rotational spectrum of $N^+_2 (B-X)(0,0)$ and of $N_2 (C-B)(0,2)$ is measured for pure helium. A best fit with simulated spectra from
Figure 2.4: Boltzmann plot for one plasma condition in argon and helium for $2 \leq J \leq 8$.

Lifbase is used to determine the rotational temperature of $N_2^+$ and a best fit with simulated spectra from Specair is used to determine the rotational temperature of $N_2$.

**Line broadening**

Besides natural broadening other effects can contribute to the broadening of emission of atoms. These broadening effects can be used to calculate plasma parameters like the electron density and the gas temperature. However, as indicated above, at atmospheric pressure, gas temperatures close to room temperature and electron densities of around $10^{20} \text{ m}^{-3}$ the van der Waals broadening has to be taken into account. This can otherwise lead to significant overestimation of the electron density [23].

**Doppler broadening**

Due to the thermal motion of the particles in the plasma, the Doppler effect results in one of the broadening contributions of atomic lines. The Doppler broadening has a Gaussian line shape with a full width at half maximum (FWHM), $\Delta \lambda_D$, of [24]:

$$\Delta \lambda_D = 7.162 \cdot 10^{-7} \lambda_0 \sqrt{\frac{T_{\text{gas}}}{M}},$$

(2.4)
2.3: Theoretical aspects and methods

with \( \lambda_0 \): wavelength in nm, \( T_{\text{gas}} \): gas temperature in K, \( M \): atomic mass of the emitter in atomic mass units.

**Stark broadening of hydrogen lines**

Due to the Coulomb interaction between the light emitting atoms and charged particles, mainly electrons, line broadening due to the Stark effect can occur. The Stark broadening results in a Lorentzian shape. In general the FWHM of the Stark broadening increases with increasing electron density [25].

To obtain the electron density the line broadening of the H\(_\alpha\) and the H\(_\beta\) lines are used. For electron densities below \( 6 \cdot 10^{20} \text{ m}^{-3} \) for H\(_\alpha\) and below \( 4 \cdot 10^{19} \text{ m}^{-3} \) for H\(_\beta\) fine structure has to be taken into account [26], as shown to be important by Bruggeman *et al.* [27]. The limit for H\(_\alpha\) is larger than for H\(_\beta\) due to the larger fine structure splitting for H\(_\alpha\) and the smaller line broadening due to the Stark effect compared to the H\(_\beta\)-line at the same electron density.

Simulations of the Stark broadening of H\(_\alpha\) for electron densities in the range of \( 1 \cdot 10^{18} \text{ m}^{-3} \) to \( 8 \cdot 10^{19} \text{ m}^{-3} \) including fine structure are used to calculate the dependency between the electron density and the FWHM. For the H\(_\beta\) line we used simulations in the range of \( 10^{18} \text{ m}^{-3} \) to \( 6 \cdot 10^{20} \text{ m}^{-3} \) by M. Á. González without inclusion of the fine structure effects [28]. To include the effects of the fine structure, these lines are folded with the fine structure of H\(_\beta\) which consists in first approximation of two components which are separated 8 pm from each other. We also corrected for the relative intensity of the fine structure components. This enables us to use the line broadening of H\(_\beta\) for \( 6 \cdot 10^{20} \text{ m}^{-3} > n_e > 1 \cdot 10^{19} \text{ m}^{-3} \).

From these fits we obtain the following FWHM relations, with \( \Delta \lambda_S \) in nm and \( n_e \) in \( \text{m}^{-3} \):

\[
\Delta \lambda_S = 1.78 \cdot \left( \frac{n_e}{10^{23} \text{m}^{-3}} \right)^{\frac{2}{3}} \text{ for } H_\alpha \text{ (double peak fit), (2.5)}
\]

\[
\Delta \lambda_S = 3.67 \cdot \left( \frac{n_e}{10^{23} \text{m}^{-3}} \right)^{\frac{2}{3}} \text{ for } H_\beta \text{ (single peak fit). (2.6)}
\]

In the case of H\(_\beta\) we obtained a FWHM formula for a single peak fit (with fine structure correction). Note that the correlation of H\(_\beta\) deviates from the standard one by 24% [25].

We also use the helium line at 667 nm to obtain the gas temperature. For this line the Stark broadening is negligible in comparison with the other contributions because of the weaker quadratic Stark effect, compared to the stronger Stark effect of the hydrogen atoms [24].
Chapter 2: Power, temperatures and electron densities of RF CAPPJs

Resonance broadening

Interactions with neutral perturbers can be categorised in two broadening effects, i.e. the resonance broadening and the van der Waals broadening.

Resonance broadening has a Lorentzian shape and occurs when the perturber and radiator are alike and either the upper or lower transition level has an allowed transition to the ground state.

The formula for the FWHM $\Delta\lambda_R$, in cm, with $N = \frac{p}{k_B T_{\text{gas}}}$ in cm$^{-3}$ and the wavelengths in cm as well,

$$\Delta\lambda_R = 8.61 \cdot 10^{-14} \left( \frac{g_1}{g_R} \right)^{\frac{1}{2}} \lambda_0^2 \lambda_R \frac{p}{k_B T_{\text{gas}}}.$$  \hfill (2.7)

For the details of the equation the reader is referred to [24]. The resonance broadening of the helium line at 667.815 nm is one of the used methods to obtain the gas temperature. Inserting all the known values one obtains:

$$\Delta\lambda_R[\text{nm}] = \frac{26.26}{T_{\text{gas}}}.$$  \hfill (2.8)

Van der Waals broadening

Van der Waals broadening is another broadening effect due to neutral perturbers. The FWHM, in cm, can be calculated with

$$\Delta\lambda_{vdW} = 8.18 \cdot 10^{-26} \lambda_0^2 \left( R^2 \right)^{\frac{3}{2}} T_{\text{gas}}^2 \cdot N \cdot \sum_i \left( \frac{\alpha_i^2 \lambda_i}{\mu_i^3} \right),$$  \hfill (2.9)

with the wavelength $\lambda_0$ in nm, $\mu$ the reduced mass in atomic mass units, the neutral particle density, $N$, in cm$^{-3}$, $i=$He or Ar and $\chi$ the fraction of the perturber. For the details the reader is referred to Yubero et al. [29].

The values for the polarizability of the perturber $\alpha$ are taken from [30] and are for helium and argon $2.05 \cdot 10^{-25}$ cm$^3$ and $1.64 \cdot 10^{-24}$ cm$^3$, respectively.

To include the fine structure in the calculation of the square radius a weighted average of the squared radii $R_i^2$ of the seven different fine structure split levels is used. After calculating the values for the case of the atomic lines, one obtains for the FWHM,

$$\Delta\lambda_{vdW}[\text{nm}] = \frac{C}{T_{\text{gas}}}.$$  \hfill (2.10)
2.3: Theoretical aspects and methods

Table 2.1: Constants for the van der Waals broadening for the different lines and gases that are used.

<table>
<thead>
<tr>
<th>Gas composition</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>He_{667nm}</td>
<td>1.79</td>
</tr>
<tr>
<td>Hα</td>
<td>2.37</td>
</tr>
<tr>
<td>Hβ</td>
<td>2.42</td>
</tr>
<tr>
<td>He-argon-mix</td>
<td>2.64</td>
</tr>
<tr>
<td>Ar</td>
<td>5.12</td>
</tr>
<tr>
<td>He-argon-mix</td>
<td>2.70</td>
</tr>
<tr>
<td>Ar</td>
<td>5.24</td>
</tr>
</tbody>
</table>

The constant C for the different lines and gases are presented in table 2.1.

Fitting procedure

The Doppler and the instrumental broadening have a Gaussian line shape. The Stark, resonance and the van der Waals broadening have a Lorentzian shape. The convolution of these two line shapes is the so-called Voigt shape with a FWHM, \( \Delta \lambda_V \), of \[ (2.11) \]

\[
\Delta \lambda_V \approx \sqrt{\left(\frac{\Delta \lambda_L}{2}\right)^2 + \Delta \lambda_S^2} + \frac{\Delta \lambda_L}{2}.
\]

Table 2.2: Calculated FWHM in nm of a helium plasma with \( T_{gas} = 400 \text{K}, p = 1 \text{atm} \) and \( n_e = 10^{20} \text{m}^{-3} \).

<table>
<thead>
<tr>
<th></th>
<th>( \Delta \lambda_D )</th>
<th>( \Delta \lambda_{instr} )</th>
<th>( \Delta \lambda_R )</th>
<th>( \Delta \lambda_S )</th>
<th>( \Delta \lambda_{vdW} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hα</td>
<td>0.0094</td>
<td>0.0081</td>
<td>0.0178</td>
<td>0.0358</td>
<td></td>
</tr>
<tr>
<td>Hβ</td>
<td>0.0067</td>
<td>0.0061</td>
<td>0.0367</td>
<td>0.0365</td>
<td></td>
</tr>
<tr>
<td>He_{667nm}</td>
<td>0.0658</td>
<td></td>
<td></td>
<td>0.0272</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2 shows calculated FWHM for pure helium for one condition. It can be seen that at these conditions van der Waals broadening is at least of the same order of magnitude as the Stark broadening for the Hα and Hβ line.

To determine the gas temperature a Voigt shape is fitted to the measured lines. The Lorentzian part of the FWHM is used to estimate the gas temperature and the electron density via the van der Waals broadening and Stark broadening in case for the hydrogen lines. For the neutral helium line the sum of the resonance and van der Waals broadening is used to calculate the gas temperature.

Figures 2.5(a) and 2.5(b) show examples of the measured line broadenings of the Hα and Hβ lines for the helium-argon mix plasma at a root mean square current.
Figure 2.5: Two fit examples from the measurements of the helium-argon mix plasma of the Voigt fit for the H\textalpha\ line and the H\beta line at $I_{rms} = 880$ mA.

(a) Two peak fit for the H\textalpha\ broadening

(b) Single peak fit for the H\beta broadening
of 900 mA. A two peak Voigt fit is used for the H\(_{\alpha}\) line including the effect of the fine structure as can be seen in figure 2.5(a). A single peak fit is used for the H\(_{\beta}\) line as explained in the section above.

**Rayleigh scattering**

The intensity of the Rayleigh scattered light is proportional to the particle density, \(n_{\text{gas}}\), and the scattering cross section, \(\sigma\). Considering the ideal gas law we obtain

\[
I \propto \sigma n_{\text{gas}} = \sigma \frac{p}{k_B T_{\text{gas}}}
\]

and

\[
\frac{I_{\text{ref}}}{I_{\text{meas}}} = \frac{T_{\text{gas}}}{T_{\text{ref}}},
\]

for the same gas composition of the reference and the measurement and a constant pressure. Note that the Rayleigh cross section \(\sigma\) for He, Ar and N\(_2\) are 74 \(\sigma_{\text{He}}\) \(\approx\) 1.12 \(\sigma_{\text{Ar}}\) \(\approx\) \(\sigma_{\text{N}_2}\) [32, 33].

The gas temperature can be obtained by measuring a reference signal with a known gas temperature and known gas composition comparing it to the signal with the unknown temperature for the same gas composition or known gas composition.

### 2.4 Results

#### 2.4.1 Power measurements

Figure 2.6 shows the power dissipation of the plasma for helium, argon and the helium-argon mix. It can be seen that for increasing current the power dissipation rises. The largest change in power is obtained for the helium discharge with up to 12 W for the highest current. A similar increase is shown for the power dissipation with the helium-argon-mixture. The power however is at the same currents lower than for helium at the higher power range.

Comparing these results with the power dissipation in argon one can see that at relatively low currents the power dissipation is higher in argon for the same current but it has a less steep slope, compared to the other cases, before it rises again until
arcing occurs (at around 1 A). In the case of helium and the helium-argon-mix discharge, arcing did not occur in the investigated current/power range.

Images of the plasmas for different applied voltages are recorded to estimate the volume of the plasma and to compare it with the power dissipation. Two examples for each gas for low and high currents, respectively, are shown in figures 2.7(a) to 2.7(f).

Figure 2.8 shows the volume estimated from the emission for a helium, an argon and an argon-helium mix plasma, respectively. It can be seen that for all cases the volume has approximately the same dependency to the \( I_{\text{rms}} \) as the power, showing that the power density is in first approximation constant for all investigated conditions as shown in figure 2.9. However in the case of helium a small decreasing trend in the power density for increasing currents is found.

### 2.4.2 Gas temperature measurements

Figures 2.10(a) to 2.10(c) show the gas temperature of the different plasmas as a function of \( I_{\text{rms}} \) obtained with the different methods as discussed in section 2.3.2. In the case of argon (figure 2.10(a)) the line broadening of the H\(_\alpha\) and the H\(_\beta\) line, with only taking into account vdW-broadening, Rayleigh scattering and the Boltzmann plot method for the rotational spectrum of OH is used for the determination of the gas temperature. Assuming that Rayleigh scattering
2.4: Results

(a) Helium plasma, $I_{\text{rms}} = 630\, \text{mA}$

(b) Helium plasma, $I_{\text{rms}} = 1090\, \text{mA}$

(c) Helium with 10% Argon, $I_{\text{rms}} = 620\, \text{mA}$

(d) Helium with 10% Argon, $I_{\text{rms}} = 1090\, \text{mA}$

(e) Argon plasma, $I_{\text{rms}} = 630\, \text{mA}$

(f) Argon plasma, $I_{\text{rms}} = 890\, \text{mA}$

Figure 2.7: Images of the plasma for 6 different investigated conditions. The outer diameter of the quartz tube is 3 mm.

is the most accurate method to obtain the gas temperature, we compared the other methods with the results obtained by this method. It can be seen, that the results obtained from the Boltzmann plot method are larger but within the error of the gas temperature obtained by Rayleigh scattering. The results from the line broadening are lower than the temperatures measured with Rayleigh scattering and the Boltzmann plot, which indicates an important contribution of the Stark broadening and a significant electron density (see next section).

Figure 2.10(b) shows the measurements of the helium-argon mix. In addition to the methods used for the pure argon case the gas temperature is also obtained from the resonance and van der Waals broadening of a neutral helium line at 667.8 nm. It shows that the temperature obtained by the helium 667.8 nm line is consistently larger than the temperature measured with Rayleigh scattering but within the margin of error. The temperature from the Hα line broadening is, in contrast to the argon case, only slightly smaller than the temperature obtained by Rayleigh scattering. The temperature obtained by the Hγ line broadening is, similar to the argon case, the smallest, which is consistent with the expected Stark broadening of these lines. The rotational temperature of the OH spectrum, however is much larger than the gas temperature obtained by Rayleigh scattering which is clearly different than in the argon case.

Figure 2.10(c) shows the results for the helium plasma. Using the Rayleigh scattering method for pure helium is very inaccurate, since the cross section of helium is much lower than the cross section of the air around and inside the plasma, as shown in section 2.3.2. Furthermore any small change in gas composition between the measurement and the reference measurement would lead to large errors...
in $T_{\text{gas}}$. Instead the rotational temperature of $N_2^+ (\text{B-X})(0,0)$ is measured. Assuming similar overestimations of the gas temperature as in case (b), the expected gas temperature is about 70 K lower than measured with the 667.8 nm line. As in the case for pure argon the H$_\alpha$ and H$_\beta$ lines underestimate the gas temperature. For the rotational temperatures obtained with N$_2^+$ and OH it can be seen that they agree but are systematically larger than the temperatures obtained with the other methods. This is also confirmed with the rotational temperature of N$_2$(C-B), which is known to be a reliable measurement of the gas temperature in helium discharges [34, 35].

Apart from the gas temperature inside the plasma, the temperature in the far effluent has been measured with a thermocouple for a pure helium and a pure argon plasma (see figure 2.11). It can be clearly seen that, for plasmas with approximately the same lengths, the gas temperature gradient outside of the plasma is much steeper for argon, than for helium. Several factors are contributing to this effect. First of all, to reach approximately the same length as the argon plasma, helium needs much more power than the argon plasma, this will contribute to the heating of the gas temperature. Furthermore, the thermal conductivity of argon, which is approximately an order of magnitude lower than the conductivity of helium, leads to a steeper temperature gradient. Note that gas temperatures below 42°C are found 2 mm away from the visible plasma tip and thus acceptable for biological treatments.

Figure 2.8: Estimated volume of the different discharges as a function of $I_{\text{rms}}$. 
2.4: Results

2.4.3 Electron density

Comparing the gas temperature obtained from the vdW broadening of \( \text{H}_\alpha \) and \( \text{H}_\beta \) in argon, helium and the helium-argon mixture, it can be seen, that the obtained temperatures are in every case lower than the temperatures obtained by other methods due to the influence of Stark broadening.

To take into account the van der Waals broadening to obtain the electron densities of the argon and argon-helium-mix plasma, the gas temperature obtained by calculated temperatures of the Rayleigh and the line broadening of the hydrogen line measurements are compared. For helium \( T_{\text{He}} = 70 \text{ K} \) is used. The difference between these temperatures is used to determine the electron density.

Figures 2.12(a) to 2.12(c) show the densities obtained by the Stark broadening. It can be seen that the electron density for argon is higher than in the helium and helium-argon-mix case. This is expected due to the lower ionisation energy of argon compared to helium [36]. The validity and accuracy of the electron density measurements is discussed in the next section.

Figure 2.9: Estimated power density of the different discharges as a function of \( I_{\text{rms}} \).
Figure 2.10: Temperature, obtained by different methods, as a function of \( I_{\text{rms}} \) for the different plasmas. The Stark effect is not considered in the calculation of the temperature from the hydrogen lines in this figure.
2.5: Discussion

As seen above, it is clear that every gas temperature diagnostic method has to be used with care. Especially in the region of interest for biomedical applications. The measurement of the gas temperature via Rayleigh scattering is, as a direct measurement of the neutral density, trustworthy, as it does not depend directly on the plasma excitation while emission spectra do. However Rayleigh measurements are not always possible, such as in the case for helium plasma in air.

Optical emission spectroscopy of the rotational band of OH(A-X)(0,0) seems to overestimate the gas temperature even though the Boltzmann plot showed no deviation of the linear slope. For the pure argon plasma, this is less pronounced and in the margin of error of the Rayleigh scattering. However it has to be noted that only low rotational numbers have been used due to the low intensity of the discharge. A potential reason for the overestimation could be the high electron temperature in these plasma jets as shown by Bruggeman et al. [37]. In [37] it has been reported that in the case of helium the non-equilibrium of the OH(A) distribution increases with increasing electron temperature.

We measured the rotational spectrum of N$_2$(C-B) and N$_2^+$(B-X) as well to obtain the gas temperature using Lifbase and Specair, assuming a Boltzmann distribution. In pure helium, N$_2^+$(B) provides higher temperatures than obtained by N$_2$(C-B) and line broadening. This is, for N$_2^+$, also observed in the work of

Figure 2.11: Gas temperature outside of the helium and argon plasma measured with a thermocouple. The tip of the plasma was approximately 1 mm away from the first measurement point in order to avoid a direct contact between plasma and thermocouple.
Figure 2.12: Electron density as a function of current root mean square for the different plasmas. The lines are shown as a guide to the eye.
Ionascut-Nedelscu et al. [38]. The obtained temperature from $N_2^+$ (B-X) is similar to the rotational temperature of OH(A-X) in the case for pure helium.

In argon and the helium-argon mix, the temperature of $N_2$(C) assuming Boltzmann distribution, which is not shown here, was typically above 850 K. This is due to the almost resonant energy transfer between metastables of argon to $N_2$(C) which strongly populates high rotational states and leads to overestimations of the gas temperature [35].

A small discrepancy between the gas temperature obtained from Rayleigh scattering and the helium line at 667 nm is observed. To calculate the gas temperature we assume that the resonance broadening and the van der Waals broadening are two independent contributions to the line broadening which is in general not true since both broadening effects originate from the same perturbing atoms. This could explain the observed discrepancy in the gas temperature measurements.

The temperatures from the Rayleigh measurements show a similar dependency of the root mean square current as the volume and the power, which is a good indication that it is a good representation of the gas temperature. Similar trends have been found in the work of Verreycken et al. for discharges of 2500 – 3500 K [15]. It has to be noted that even though the temperatures range from 300 to 600 K, depending on the gas, the gas temperature at the end of the jet is smaller than the temperature in the active region of the plasma as is estimated from gas temperature measurements by temperature strips and thermocouples. These measurements yield temperatures between 300 to 350 K at the tip of the plasma at powers of around 1 Watt or a few millimetres away from the plasma for argon, while for helium, at the same length of the visible plasma tip, the temperature drops slower. This shows that in spite of the reported temperatures up to 600 K in the core the APPJ can be used for heat sensitive applications.

The obtained rotational temperature of OH(A) always seems the largest compared to the other temperature measurements. We now estimate the gas temperatures upper limit from a simple power balance of the plasma, assuming that all power is converted in gas heating and all heat is removed by the forced flow.

This leads to

$$P = \frac{\Delta m}{\Delta t} c \cdot \Delta T,$$

with the power $P = 3$ W, the heat capacity $c = 5193 \frac{J}{kgK}$ and the mass flow $\frac{\Delta m}{\Delta t} = 3 \cdot 10^{-6} kg/s$. This shows that the gas temperature cannot exceed 500 K at 3W which corresponds with 800 mA in helium, while the rotational temperature of OH(A) is $(600 \pm 50)K$ in this case. It is thus clearly an overestimate of the gas temperature for the helium plasma.

Measurements of the electron density clearly show, that $n_{eHe} < n_{eAr}$. In all cases the electron density is approximately constant for all currents, which is to be
expected since the power density is in first order constant. Furthermore the strong influence of the van der Waals broadening is clear, as for the reported conditions the van der Waals broadening is of the same order of magnitude or larger than the Stark broadening. This means that the accuracy of the electron density measured with Stark broadening is determined by the accuracy of the gas temperature since:

$$\Delta \lambda_S = \Delta \lambda_L = \left( \frac{C}{T_0^{0.7}} \right).$$ \hspace{1cm} (2.15)

This leads to, e.g. for the H$_\alpha$ in helium at $T = 400$ K with an error of $\Delta T = 100$ K to an uncertainty of 4 pm for the FWHM of the Voigt fit which corresponds to an uncertainty of $\Delta n_e \approx 2.5 \cdot 10^{19}$ m$^{-3}$.

Clearly only an order of magnitude estimation can be reached for the electron densities for low temperature atmospheric pressure plasmas with this method. For low ionized plasmas such as the helium discharge the presented results are an upper limit.

Since the main electron energy loss is due to elastic collisions in the plasma jet, a simple power balance equation can be used to get an independent estimation of the electron density with

$$\frac{P}{V} \approx n_e \cdot n_{He} \cdot \frac{3}{2} k_{el} \cdot (T_e - T_g) \cdot 2 \cdot \frac{m_e}{m_{He}}.$$ \hspace{1cm} (2.16)

Using an elastic collision rate, $k_{el}$, of $0.6 \cdot 10^{-13}$ m$^3$/s obtained from Bolsig+ \[39\], a helium density, $n_{He}$, of $2.5 \cdot 10^{25}$ m$^{-3}$ and an estimated electron temperature, $T_e$, of 3 eV the formula can be rewritten as

$$\frac{P}{V} \approx 2.48 \cdot 10^{-10} n_e.$$ \hspace{1cm} (2.17)

With an obtained power density of $(1.2 \pm 0.4) \cdot 10^9 \frac{W}{m^2}$ the estimated electron density is

$$n_e = (4.0 \pm 1.3) \cdot 10^{18} m^{-3}.$$ \hspace{1cm} (2.18)

This shows that the estimate of $n_e$ obtained by a power balance seems to be more accurate than the line broadening measurements. An additional contribution to the experimentally obtained line broadening can be induced by the electrical field in the plasma (see e.g. \[40\]). This could be especially important in the case of helium. The Holtsmark electrical field \[6\] for $n_e$ smaller than $10^{-19}$ m$^{-3}$ is equal
or smaller than $100 \frac{V}{cm}$. The electrical field in a helium discharge of $3 \text{eV}$ is in the local field approximation about $250 \frac{V}{cm}$ [39] thus in the same order of magnitude as the Holtsmark electric field.

Comparing the shape of the different plasmas it is clear that the argon discharge is more radially contracted than the helium discharge. In many cases thermal ionization instabilities can explain these contractions [41, 42]. However, for thermal instabilities the temperature in the bulk is in the order of thousand Kelvin or more. Considering the obtained gas temperatures of the plasma source thermal instabilities are unlikely.

We compare the time scales of diffusion and dissociative recombination including helium, argon, nitrogen and oxygen. The timescale of dissociative recombination is estimated with

$$\tau_{\text{diss}} = \frac{1}{n_e k_{ei}}, \quad (2.19)$$

with $k_{ei}$ being the electron-ion recombination, taken from [43]. The timescale of diffusion is estimated with

$$\tau_{\text{diff}} = \frac{\Lambda^2}{D}, \quad (2.20)$$

with $\Lambda = \frac{R}{2} \approx 0.1 \text{mm}$ being the geometric constant with $R$ being the radius of the plasma, $D = \frac{\mu kT_e}{e}$ the diffusion, with $kT_e$ assumed to be maximally $3 \text{ eV}$ as the electron temperature and $\mu$ as the ion mobility taken from Ellis et al. [44].

Table 2.3: Timescales of diffusion and dissociative recombination.

<table>
<thead>
<tr>
<th>Gas</th>
<th>$t_{\text{diss}}[s]$</th>
<th>$t_{\text{diff}}[s]$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Helium</td>
<td>$10^{-6} - 10^{-5}$</td>
<td>$10^{-6}$</td>
</tr>
<tr>
<td>Argon</td>
<td>$10^{-8}$</td>
<td>$10^{-8}$</td>
</tr>
</tbody>
</table>

Table 2.3 shows the results of the calculation. The estimates are valid for the following ions: $N_2^+$, $O_2^+$, $He_2^+$ and $Ar_2^+$. It shows that, in the case of argon the dissociative recombination is much faster than the diffusion. The local electron loss results in a contracted shape of the discharge in the case of argon is a necessary condition for contraction. In the case of the helium discharge diffusion losses are faster or equal to recombination losses, which is in agreement with the more diffuse shape of the plasma. A similar effect is also recently reported in atmospheric pressure microwave plasmas [45].

2.6 Conclusion

We obtained different plasma parameters of a helium and an argon cold APPJ operating in open air. The power dissipation of the plasma was measured with
electrical probes, with corrections of the power dissipation in the matching box. The power dissipation ranged from 100 mW to 12 W for the pure helium plasma and up to 6 W in the case of the argon plasma. It was shown that the discharges in the investigated range had in first approximation a constant power density.

The gas temperature was obtained by Rayleigh scattering, the rotational spectrum of the OH(A-X)(0,0) transition and line broadening. The rotational temperature of OH and N\textsubscript{2}\textsuperscript{+} seemed, in the case of helium and the helium-argon mixture, to be an overestimation of the gas temperature, while rotational temperature of N\textsubscript{2}(C-B). The measured gas temperature of the helium plasma ranged between 300 K to 600 K for different $I_{\text{rms}}$ from 500 to 1100 mA. The gas temperature of the argon plasma was in the range of 380 K to 450 K.

The Stark broadening of H\textsubscript{a} and H\textsubscript{\beta} was used to estimate the electron density of the discharges. The results showed that the helium plasma had a density of around $(3.4 \pm 2.5) \cdot 10^{19} \text{ m}^{-3}$ while in the argon discharge the value was around $(1.1 \pm 0.7) \cdot 10^{20} \text{ m}^{-3}$. A power balance estimate in the case of the helium plasma provided a more accurate electron density of $(4.0 \pm 1.3) \cdot 10^{18} \text{ m}^{-3}$ compared to the line broadening method due to the important contribution in the experimental line profile of the van der Waals broadening and inaccuracy in the gas temperature.

With the obtained results the radial contraction of the argon plasma, compared to the more diffuse helium plasma, was explained by determining the dominant charge loss mechanisms. It had been shown that dissociative recombination processes occur much faster than diffusion, hence leading to a more contracted plasma in argon. For helium charge losses are dominantly diffusive which is consistent with the more diffuse shape of the helium discharge.
References


Chapter 3

Characteristics of continuous and time modulated RF plasmas in contact with conductive and dielectric substrates *

Abstract

In this chapter the influence of substrates of different conductivity and permittivity in direct contact with three different operational modes of atmospheric pressure RF plasma jets is investigated. Two different electrode configurations (creating either a linear or a cross electric field) and, for the linear field configuration, two voltage modulations (continuous RF and kHz pulsed RF) have been studied. Electrical and optical diagnostic methods have been performed in order to get quantitative data on the change in plasma dissipated power and gas temperature, when the plasma is in direct contact with the substrate. In all three investigated cases the power dissipation and gas temperature, significantly increase when the plasma is in direct contact with a conductive substrate. The increase of power is attributed to a change of the equivalent electrical circuit, leading to a more favourable matching between the input power and the plasma source.

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3.1 Introduction

Depending on the type of discharge and the application, the plasma is either in direct contact with the treated substrate or not. Except for surface DBDs and coplanar DBDs (see e.g. [3, 4]), for most applications based on dielectric barrier discharges the treated substrate acts as one of the electrodes and is needed to create the plasma [1–3]. The disadvantage of those sources is that the plasma itself depends strongly on the characteristics of the substrate, such as conductivity, permittivity and smoothness of the treated area, which makes diagnostics of plasma parameters challenging [5].

In a plasma jet, however, the plasma is in most cases remotely created between two electrodes in a noble gas [6, 7]. Due to the electric field and the gas flow, the plasma created species are then forced out of the plasma zone in the tube into the open air. The advantage with these devices is that the treated substrate is not needed in order to sustain the discharge, which means that the plasma can either directly (in contact) or indirectly treat the material.

It is commonly believed that the main difference between a direct and indirect treatment is that in the latter case, only long living species are able to reach the substrate, while in the first case also charged species, (V)UV and the electric field will interact with the substrate [8]. However, as in the case of a dielectric barrier discharge, the moment the plasma is close to or in contact with a substrate, the substrate itself will act as an additional electrode, changing the electric circuit and thereby altering the plasma properties itself. How much the plasma changes depends on the conductivity of the substrate and the configuration of the plasma source [9–12].

In most cases the influence of the substrate on the plasma itself has been investigated using imaging. Results indicate that a plasma in contact with an insulator shows no significant change of the plasma while touching a conductive substrate does increase the intensity of the emission and in some cases the length of the plasma jet plume [13].

Simulations of a helium plasma needle show that particle fluxes depend strongly on the treated substrate. The reason for that is the altered configuration of the electric field which will change the flux of charged particles onto the substrate [11]. Experimentally these simulations have been compared with images of the visible plasma jet and power measurements as a function of the distance between the plasma and the treated substrate, in this case agar (a gel-like solid surface used for bacteria and fungi growth) [10, 14].

Molecular beam mass spectrometry which usually samples the plasma with a metal orifice will change the plasma properties completely from the free plasma and the results obtained from these experiments should be interpreted with care as observed and mentioned e.g. in [15, 16].
3.2: Setup and methods

As in most _in vitro_ biological treatments water and saline-like solutions are present, understanding how the plasma behaves in contact with liquids is very important.

**Table 3.1:** Typical electrical conductivity (\(\sigma\)) and relative permittivity (\(\varepsilon_r\)) values of the substrates used in this work. [*] measured.

<table>
<thead>
<tr>
<th>substrate</th>
<th>(\sigma) [S/m]</th>
<th>(\varepsilon_r)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tap water</td>
<td>0.025[*]</td>
<td>80, distilled at 1.6 GHz and 20°C [17]</td>
</tr>
<tr>
<td>aluminium</td>
<td>(1.4) (\cdot) 10(^7) [18]</td>
<td>-</td>
</tr>
<tr>
<td>glass (BK7)</td>
<td>(10^{-11}) to (10^{-10}) [12]</td>
<td>4.9 at 1 MHz [19]</td>
</tr>
</tbody>
</table>

In this work we apply _in situ_ measurements of the plasma dissipated power and the gas temperature, when the plasma is in contact with a substrate, i.e. water, glass and metal. The typical values of permittivity and conductivity of the interfaces used are shown in table 3.1. The observed parameters have been obtained by time resolved ICCD imaging and time resolved plasma dissipated power measurements combined with spatially resolved optical emission spectroscopy. An RF argon plasma jet with two different electrode configurations and time modulated and continuous RF excitation has been used.

The chapter is structured as followed. In section 3.2 the plasma source and the diagnostics are introduced. Section 3.3 deals with the results starting with obtained CCD images, followed by electrical characteristics and ICCD images (section 3.4) and gas temperature measurements of the plasma in contact with water (section 3.5). In addition, these results are compared with measurements performed with a glass and an aluminium substrate (section 3.6). In section 3.7 a summary of the results is given and we discuss the consequences of the presented research for related studies.

### 3.2 Setup and methods

Figure 3.1 shows a schematic view of the setup. An RF voltage with a frequency of 13.56 MHz generated with a waveform generator (Agilent 33220A) and amplified by a power amplifier (Amplifier Research 75AP250) is applied to the needle electrode. The output of the amplifier is connected to an inductance, which is close to resonance with the jet at a frequency of 13.56 MHz. This simple network design has been chosen in order to reduce the amount of impedances in the circuit connected to ground. These need to be otherwise considered when correcting for the power which is dissipating in the matching network (see power measurements below and chapter 2). The matching network is connected to the plasma source, which consists of a needle electrode (\(\varnothing=1\) mm) in the centre of a dielectric enclosing. The needle is surrounded by a quartz glass tube, with an inner diameter of 1.7 mm and an outer diameter of 3 mm. An argon flow of 1 slm through the glass tube is controlled by a mass flow controller (Brooks 5850E calibrated for 0-3 slm...
of helium) connected to a control unit (Brooks 0254).

For the grounded electrode either a ring electrode surrounding the glass tube or a plate electrode in front of the glass tube is used. These configurations result in either an electric field perpendicular (cross-field configuration) or parallel to the gas flow (linear-field configuration) [20]. The ring electrode has a length of 5 mm, the end of the ring electrode is 2 mm behind the needle electrode and 0.5 mm before the end of the glass tube. The plate electrode is at a distance of 4 mm away from the needle electrode and 2 mm away from the end of the glass tube. The plate has a hole with a diameter of 5 mm to let the gas flow and the plasma penetrate through it.

In order to reduce the gas temperature without increasing the gas flow and keeping a plasma length of a few millimetres we use a second waveform generator (Agilent 33220A) and apply a 20 kHz modulation of the RF voltage with a duty cycle of 20% when using the ring electrode configuration. The plate electrode is used either in continuous mode or with the same pulse modulation as with the ring electrode.

In order to compare the three sources at least one parameter needs to be comparable for all three configurations. Voltage and therefore power dissipation could not be used, since the power coupling between these devices depends on the electrode configuration which changes the matching. If the same power for the continuous plate electrode is used as for the pulsed ring electrode, either the plasma is too long and too hot in the former case, or the voltage is too low to sustain a plasma in the latter case. To resolve this issue the input parameters (i.e. amplifier gain and RF voltage to the amplifier) are chosen in order to obtain the same plasma length measured between the grounded electrode and the end of the visible plasma plume, i.e. 5 – 6 mm, while maintaining a gas temperature at the tip of the plasma of below 100 °C.

To measure changes in the plasma induced by a substrate, different materials
have been placed below the plasma jet. The distance between plasma plume and substrate has been decreased until a clear visible connection between the plasma and the substrate is observed. To maintain a stable contact, the distance between the substrate and the jet is further decreased by 2 mm in all cases. The gain of the amplifier and the RF voltage supplied to the amplifier have been kept the same between the "free" plasma and the plasma in contact with a substrate. A CCD-camera (Sony DSC-R1) has been used to obtain images of the free plasma comparing it with a plasma in contact with a substrate.

Figure 3.2: Equivalent circuit of the two electrode configuration, i.e. plate (a) and ring (b) electrode.

Figure 3.2 shows a simplified equivalent circuit for the plate (3.2(a)) and the ring electrode configuration (3.2(b)). The amplifier is represented by an alternating voltage source and a 50 Ω resistor. The matching network (a coil) is described as an inductance and a resistor, which includes the resistance of the cable and of the needle electrode. In the case of the plate electrode a plasma is created between the needle electrode and the substrate (if present), which are described as a capacity and an impedance, respectively. The latter can be mainly resistive or capacitive, depending on the substrate (see table 3.1). Since there is no plasma
between the needle and the plate electrode, this can be viewed as an air capacitor.

The main difference between the plate and the ring electrode configuration is that a plasma is not only created between needle and substrate but also created between the needle and the glass tube which is surrounded by the grounded ring electrode. This system is described as a resistor and a capacitor in series, which is in parallel with the plasma connecting to the substrate.

When no substrate is present, the plasma is capacitively coupled to its surroundings and $Z_{sub}$ is a capacitor.

### 3.2.1 Power and phase shift measurements

A voltage probe (Tektronix P5100) and a current probe (Pearson 2877) connected to an oscilloscope (Agilent Technologies DSO1024A) have been connected between the power amplifier and the matching network to measure the voltage across and the current through both the matching box and the plasma source.

![Figure 3.3: Voltage and current during the pulse modulation with the ring electrode configuration without plasma.](image)

Figure 3.3 shows an example of the voltage and current obtained for the ring electrode configuration in pulsed mode without a gas flow, i.e. without a plasma. With $P = \frac{1}{7} \int_{t_0}^{t_0+\frac{T}{2}} U(t) \cdot I(t) dt$, the average power over one RF cycle is then calculated. In order to obtain the power dissipated only in the plasma the delay induced by the voltage and current probes and the oscilloscope have to be corrected \[21\] as well as the power dissipated by matching network. Details can be found in chapter 2.

Figure 3.4 shows an example of the instantaneous power, i.e. $P(t) = I(t) \cdot U(t)$
and the power dissipation averaged over one RF cycle of the circuit without an applied gas flow (no plasma) calculated from the voltage and current signal from figure 3.3. Two peaks in power are visible at the beginning and the end of the pulse. These peaks indicate that energy is stored in parts of the electrical circuit and released at the end of the pulse. Calculating the energy of the peaks results in an energy of 6.76 µJ and 4.10 µJ for the first and the last peak, respectively. The constant positive power offset in between the peaks originates from the resistance of the electrical parts, most dominantly from the wire of the coil in the matching network. The average power loss in the resistive part of the setup is (2.86 ± 0.12) W for the settings of figure 3.4. Similar results are obtained with the plate electrode.

Next to the power dissipation the phase shift between the voltage and current signal has also been obtained to monitor the change in impedance of the circuit. The obtained phase shift is the phase induced by the coil of the matching network, the jet geometry, the plasma and any present stray capacitances.

For the continuous plate electrode the power measurements have been validated with power measurements performed with a power meter (AR DC2600A), showing that the calculated average power corresponds to the measured forward power subtracted with the reflective power.

Note that for the time resolved ICCD images a different power amplifier is used (E & I A-075) and the matching frequency shifted from 13.56 MHz to 13.96 MHz.
3.2.2 Time resolved ICCD images

An ICCD camera (Stanford Computer Optics 4Picos) has been used and synchronised with the kHz pulse modulation to obtain time resolved images of the plasma at different delay times during the 50 µs period of the modulated RF power. The exposure time of the images was set to 100 ns. The images are the accumulations of 1000 consecutive pulses at the same delay time. The images are synchronised with the pulse modulation but not with the RF frequency, meaning that each accumulation was obtained at a random phase of the RF cycle. This results in a measured plasma emission averaged over the RF modulation. In order to increase the spatial resolution of the image a camera lens (Tamron Japan 572D) and an additional lens with a focus of 30 cm have been placed between the ICCD camera and the plasma source. The images are wavelength integrated over the visible wavelength range.

3.2.3 Spatially resolved gas temperature

![Figure 3.5: Example of the spectrum from the OH emission band for the ring electrode configuration with no substrate approximately 5 mm away from the needle electrode. Plasma dissipated power = 5.2 W.](image)

A 1 m-monochromator (Jobin Yvon HR 1000 M) has been used to obtain the molecular emission band of the OH(A-X)(0,0) transition. The monochromator is backed by a CCD camera (SBIG ST-2000XMI-UV, pixel size 8 µm). Two quartz lenses (f = 30 cm) have been aligned between the plasma jet and the monochromator to project a 1 to 1 image of the plasma jet on the entrance slit of
the monochromator. An example of the emission spectrum can be seen in figure 3.5 showing a normalised emission spectrum averaged axially over 30 pixels.

Figure 3.6: Example of the Boltzmann plot from the OH emission band of the spectrum shown in figure 3.5.

An example of the Boltzmann plot method corresponding to figure 3.5 can be seen in figure 3.6. Only the first five rotational numbers of the rotational emission band have been considered (corresponding to 6 different transitions), since it has been shown by Bruggeman et al. [22] that emission lines from higher rotational numbers do not necessarily follow a thermalised Boltzmann distribution corresponding to the temperature of the gas. In chapter 2, we have validated this method with Rayleigh scattering. The error indicated in figure 3.16 only takes into account the error of the least squares linear fit applied to the data.

The power shown in section 3.4.3 and the temperature shown in section 3.5 have been measured simultaneously. The CCD images have been captured at the same conditions but not during the same measurement series.

3.3 Visual appearance of the plasma jets

3.3.1 CCD-images

Figure 3.7 shows images of the plasma in the three different configurations. The first image 3.7(a) is the continuously driven RF plate electrode configuration. As
Chapter 3: Continuous and time modulated RF CAPPJs

(a) Continuous RF driven jet with grounded plate electrode. (b) Modulated RF driven jet with grounded plate electrode. (c) Modulated RF driven jet with grounded ring electrode.

Figure 3.7: CCD images of the three different plasma conditions not in contact with water (free plasma). The dashed line indicates the position of the grounded plate electrode.

(a) Continuous RF driven jet (b) Modulated RF driven jet (c) Modulated RF driven jet with grounded plate electrode. with grounded plate electrode. with grounded ring electrode.

Figure 3.8: CCD images of the three different plasma conditions in contact with water (touching water). The upper dashed lines indicate the position of the grounded plate electrode, if present. The lower dashed lines indicate the position of the water substrate.

seen here, previously in chapter 2 and in [23], the plasma is contracted showing either one stable column or one or more randomly moving filaments. As explained in chapter 2 and [24], losses dominated by dissociative recombination compared to losses due to diffusion are the main cause for the contraction of the plasma. The production of metastables in the dissociative recombination process leads to high densities of metastables and an efficient ionisation in the centre, with

\[
\begin{align*}
\text{Ar}_m^+ + e & \rightarrow \text{Ar}_m + \text{Ar} \\
\text{Ar}_m + e & \rightarrow \text{Ar}_m^+ + 2e
\end{align*}
\] (3.1) (3.2)

The randomly created filamentation of the plasma as seen in figure 3.7(a) is contributed to a relatively high absorbed power in the plasma which is then distributed into two or more randomly moving filaments rather than into one stable plasma column. However, the geometry, the high electric field at the needle tip and the presence of the dielectric glass tube also play a role in the filamentation and the stability of the plasma. Filamentation in RF plasma jet sources have been also observed elsewhere and can exhibit self organized behaviour [25].
In figure 3.7(b) the same electrode configuration is used but the RF signal is time modulated at 20 kHz and 20 \% duty cycle. In order to sustain the plasma and keep the length the same as in the continuous RF case, the power has to be increased in the case of the modulation. This leads to a higher instantaneous power in the latter case, but the average power and the gas temperature is lower than for the continuous RF driven jet (see section 3.4.3 and section 3.5).

The reason for the plasma showing only one filament when the voltage is time modulated, is probably due to the gas flow and the fact that the plasma is off every 50 $\mu$s for 40 $\mu$s, leaving time for the charge in the vicinity of the needle to significantly reduce and metastable species to be blown out. This leads to a reduction in the ionisation rate at the needle tip and a preferential direction guided by the gas (and metastable) flow. This has also been observed in the case of a pulsed DC excited plasma at similar frequencies as will be shown in chapter 7. The advantage of the time modulation is that the relative high electron and reactive species density (typically in RF plasmas) is present, while having a lower gas temperature due to the low duty cycle (20 \%) (see also e.g. [26–28]).

The last image, figure 3.7(c) shows the plasma created with the grounded ring electrode configuration. The modulation of the plasma is the same as for the grounded plate electrode configuration. With a gas flow of 1 slm and a strong discharge between the needle and the glass tube surrounded by the grounded ring electrode, the modulation is necessary to prevent the gas from heating up too much (inside the glass tube), while maintaining a plasma length of a few millimetres outside of the tube. Most of the plasma is created inside the glass tube in between the needle and the grounded ring, since the electric field is the highest in this region and drops rapidly outside the tube. Due to the gas flow this plasma is then blown out into the ambient air, resulting in a more homogeneous and diffuse plasma compared to the plasmas created with the plate electrode.

In the bulk of the diffuse plasma a bright core can be seen which is created, as in the previous two cases, due to the high electric field in the axial direction caused by the sharp needle tip. Note that, if the gas flow rate is higher and becomes turbulent, the filament disappears and the length of the diffuse plasma is also shorter, similar to [29, 30] and other references therein. The low gas flow of 1 slm is chosen in order to perform treatment of liquid in small volumes with the plasma source and to keep a non-turbulent gas flow.

If a conductive substrate, such as water, is introduced to the circuit, it can be seen that, if the substrate is close enough, the length of the plasma is increasing towards the substrate in all three cases. If the distance between plasma and substrate is even smaller, the plasma gets in direct contact with the substrate as shown in figure 3.8. For these images and the following measurements tap water with a conductivity of 250 $\mu$S/cm is used. The water is brought into contact with the plasma by decreasing the distance between plasma and water while keeping the input parameters of the RF amplifier constant. Liquids ranging from distilled water to NaCl-solutions with a conductivity of up to 14.2 mS/cm have been used.
as well. However, for the investigated conditions of this work no significant dependence of the conductivity of the water in power dissipation and temperature have been observed. Figure 3.8(a) shows the plasma with the grounded plate electrode with continuous RF excitation in direct contact with a water substrate. Similar images have also been obtained for other plasma sources [13, 25].

For the time modulated cases the plasma changes into a more intense filament (figure 3.8(b) and figure 3.8(c)). In the case of the modulated ring electrode configuration (figure 3.8(c)), a strong, contracted discharge column is clearly visible between the needle electrode and the water substrate, while a diffuse discharge is observed without the liquid. Both filaments in figure 3.8(b) and figure 3.8(c) are stable and are not moving randomly on the substrate as in the case for the continuously driven plasma.

Figure 3.9: Comparison of the emission spectrum of the ring electrode plasma (pulsed) and the plate electrode plasma (pulsed and continuous) for the same peak power settings as used for the temperature measurements (free plasma). An intensity offset of 0.25 and 0.5 has been added to the spectra obtained from the plate electrode, respectively.

Figure 3.9 shows an emission spectrum of the plasma, obtained coaxially, created with the ring and the plate electrode configuration at the same peak dissipated power between 200 and 1000 nm. The spectrum is normalized to the highest peak intensity and is not corrected for the wavelength dependent sensitivity of the spectrometer.

The emission spectrum in the UV range is dominated by $N_2$(C-B) in the range from 300 to 400 nm and OH(A-X)(0,0) emission at 310 nm. The ratio between the nitrogen emission and the OH peak is higher in case of the plate electrode (and highest for the continuous RF). This is to be expected since the majority
of the plasma for the ring electrode configuration is created inside the glass tube (less amount of air in the plasma plume). The plate electrode plasma is sustained outside the tube, where the air concentration in the argon effluent is higher [31]. This leads to a higher emission of the nitrogen bands. Below 300 nm rotational bands of the NO(γ)-system are visible in the case of the plate electrode configuration. In the range between 500 to 1000 nm atomic line emission from argon and the emission line from atomic oxygen at 777 nm are the most dominant peaks.

### 3.4 Electrical and optical characteristics

The main cause for the change of the plasma when a substrate is introduced is due to a change of electric field. This results in a change in plasma impedance, altering the load of the amplifier and therefore changing the power delivered into the plasma. These effects are investigated in this section in detail by analysing the voltage, current, phase shift and power of the plasma.

#### 3.4.1 Time resolved power and ICCD images

![Figure 3.10](image)

**Figure 3.10:** Time resolved power dissipation over period of the RF modulation with and without direct contact with the water substrate at 6 µs. The jet configuration is with a grounded plate electrode.

In figure 3.10 the time resolved average power over one RF cycle is obtained for the time modulated plate electrode configuration with the plasma switched on. In
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this figure the power is shown for a plasma with and without direct contact with the water substrate. As explained above, the power is corrected for the power dissipation due to resistances in the electrical network. The power is not corrected for the reactive part contributed by the capacitive behaviour of the circuit (the power peaks during the on and off switching of the RF power).

For these measurements the input power as well as the distance between plasma source and water substrate have been adjusted so that the plasma is in direct contact with the water substrate approximately half of the time. The point in time when the propagating plasma reaches the water substrate is determined by taking time resolved ICCD images of the plasma (figure 3.11) and linking the time delay of the ICCD camera to the corresponding time of the current-voltage waveforms. For these measurements the time at which the plasma reaches the water substrate is around \((6 \pm 0.5) \mu s\) after the start of the pulse as indicated in figure 3.10 by the shaded area.

The time 0 \(\mu s\) corresponds to the start of the trigger to switch on the RF voltage as shown in figure 3.3. The discharge ignites at the plasma tip at a delay of 1.5 \(\mu s\). This is in correspondence with [32]. The voltage pulses switches off after 10 \(\mu s\) (see also figure 3.4 or figure 3.10). For the free plasma a weak emission is still visible of up to 1 \(\mu s\) after the plasma is switched off.

Figure 3.11(b) shows the time resolved images of the same plasma setting, when in contact with the water substrate. Again, the plasma ignites around 1.5 \(\mu s\) after
3.4: Electrical and optical characteristics

the start of the pulse and is switched off at a delay of 10 µs.

Looking at the dissipated power of the free plasma it can be seen that there is a linear increase in power over roughly the first 4 µs starting from 2.7 W with a slope of (0.82 ± 0.03) W/µs before the power stays constant at 5.7 W, for these settings. In the case of the touching plasma it is clearly visible that the moment the plasma is in contact with the substrate a second significant steep increase in power, up to a factor of 2.5, is observed. Before the plasma reaches the water substrate the power dissipation in the plasma is the same as in the case when no substrate is exposed to the plasma source. Comparing this with the corresponding ICCD images a clear increase of intensity is also visible when the plasma is connecting to the water substrate (visible in figure 3.11 at 8 µs).

Further, from the ICCD images in figure 3.11 it can be seen that the recombination lifetime is longer when the plasma is in contact with the liquid, since the plasma emission vanishes at around 12.5 µs instead after 11 µs as in the case of the free plasma. The higher intensity and the longer lifetime indicate that in these cases the electron density and/or the amount of metastables increases, when the plasma is in direct contact with the liquid.

Note that a long afterglow in the emission of NO(A-X) is observed in [32], due to the fact that the NO (A) state is produced by \( \text{N}_2(A) + \text{NO}(X) \rightarrow \text{NO}(A) + \text{N}_2(X) \). The decay is a measure of the metastable lifetime of \( \text{N}_2(A) \) rather than the effective radiative lifetime of NO (A). A higher density of metastables, when the plasma is in contact with the liquid could thus also contribute to the strong emission and the longer afterglow as observed in figure 3.11(b).

In figure 3.12 the power dissipation of the ring electrode configuration is shown. In order to get to a similar length in plasma as in the case presented in figure 3.10 and the time at which the plasma touches the water equal to 6 µs, the power is chosen to be higher than in the case of the plate electrode.

As can be seen in figure 3.13 the power of the plasma increases as well when the plasma makes contact with the water substrate. However this increase of dissipated power does not exceed a factor of 1.3 and is thus less pronounced compared to the plate electrode geometry.

Comparing the rise time of the time resolved power between the time modulated plate electrode and the pulsed ring electrode configuration, it can be seen that the power remains constant after 2 µs for the latter case. In the case of the plate electrode geometry, the power increases up to 6 µs before reaching a steady state value. The difference in rise time can be attributed to the difference in gap size between needle and grounded electrode, which is approximately a factor 6 larger in the case of the plate electrode configuration.

Time resolved ICCD images have been obtained for the ring electrode configuration and are shown in figure 3.13. The ignition of the ring electrode plasma happens 0.5 µs earlier than for the plate electrode configuration. However, the
electric field is much higher in case of the ring electrode, due to the much smaller distance between the powered and the grounded electrode. Again, a large increase in intensity is observed, the moment the plasma connects to the water surface. The emission is also sustained for a longer time after the RF voltage is switched off compared to the free plasma.

### 3.4.2 Increase of plasma length during the pulse

From the ICCD images the propagation in the four different plasma configurations from the beginning of the pulse until the plasma is in direct contact with the liquid substrate, i.e. after a delay of $6 \mu s$, has been calculated and is shown in figure 3.14.

Note that the velocity does not correspond to the velocity of the ionisation front during an RF cycle, which will be at least one order of magnitude faster [32]. Furthermore, the gas flow is significantly smaller and in the order of $10 \text{ m/s}$. The images therefore show the slow expansion of the plasma over many RF cycles. The increase of length of the plasma plume during the time modulation can be explained by pre-ionisation linked with the number of RF cycles at the specific time point during a single kHz period [33].

In the case of the plate electrode configuration it can be seen that the velocity during the first $3 \mu s$ increases when the liquid layer is in the vicinity of the plasma. The peak velocity is reached, both with and without the liquid, when the plasma
3.4: Electrical and optical characteristics

Figure 3.13: Time resolved images of the pulse-modulated grounded ring electrode configuration. The images correspond to the experimental conditions of figure 3.12.

is at the position of the plate electrode.

The velocity in the case of the the ring electrode configuration is during the first 4 \( \mu s \) a factor 2 to 3 smaller than in the case of the plate electrode. The higher velocity of the tip of the plasma is expected due to the electric field parallel to the gas flow. The velocity is larger when the plasma propagates towards the liquid substrate, compared to the free plasma.

### 3.4.3 Voltage, current and power during one RF cycle

Table 3.2: Plasma dissipated power, apparent power and phase shift of the six different conditions investigated.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Plasma power [W]</th>
<th>App. power [W]</th>
<th>Phase [°]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plate (cont.) free</td>
<td>1.1 ± 0.2</td>
<td>14.4 ± 0.5</td>
<td>77 ± 1</td>
</tr>
<tr>
<td>Plate (cont.) touching</td>
<td>2.9 ± 0.2</td>
<td>10.1 ± 0.4</td>
<td>63 ± 4</td>
</tr>
<tr>
<td>Plate (mod.) free</td>
<td>2.7 ± 0.2</td>
<td>13.5 ± 0.5</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>Plate (mod.) touching</td>
<td>6.0 ± 0.2</td>
<td>10.0 ± 0.4</td>
<td>34 ± 1</td>
</tr>
<tr>
<td>Ring (mod.) free</td>
<td>5.2 ± 0.1</td>
<td>7.6 ± 0.4</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>Ring (mod.) touching</td>
<td>6.2 ± 0.1</td>
<td>8.5 ± 0.4</td>
<td>26 ± 7</td>
</tr>
</tbody>
</table>

To further investigate the influence of the conductive substrate on electrical pa-
Chapter 3: Continuous and time modulated RF CAPPJs

Figure 3.14: Velocity of the visible plasma tip as a function of time during one modulation cycle for the different plasma electrode configurations either with or without a water substrate. The plasma reaches the water substrate at 6 µs. The accuracy for all velocities, which depends on the spatial resolution of the images, is about 0.8 km/s as indicated for one data point.

Parameters, voltage, current and phase shift between those signals have been compared for the different conditions.

Figure 3.15 shows one RF cycle of the voltage and current signals of the three plasma sources either in touching or non-touching mode. The calculated dissipated power in the plasma and phase between the voltage and current are indicated in the graphs. The accuracy of the power is determined by the systematic error due to the calculation of the phase shift originating from the uncertainty in the time delay measurement of the cables and the U-I-measurement. The uncertainty of the cable delay is calculated to be in the order of 0.5 ns. The statistical error is calculated by calculating the power over around 20 RF cycles and is not higher than 5 %.

Since the power depends on the phase, the closer the latter gets to zero, the smaller is the error due to the the cable delay. The phase is measured by determining the time difference between the zero crossings of the voltage and current for 20 RF cycles.

Figure 3.15(a) shows the voltage and current waveforms for the continuous driven RF plasma with the plate electrode. The power and phase between voltage and current are indicated in the graphs. Comparing the voltage and current waveforms of the touching and non-touching mode, it can be seen that the signal is changing
3.4: Electrical and optical characteristics

Figure 3.15: Voltage and current waveforms of the three different investigated settings for touching and non-touching mode.
in voltage, current amplitude and phase shift.

The apparent power, \( P_{\text{app}} = U_{\text{rms}} \cdot I_{\text{rms}} \), is decreasing, when the plasma is in contact with the water substrate. But as the phase shift is decreasing, a more favourable matching between power amplifier and plasma source occurs, i.e. plasma and matching network impedance approaches the impedance of the amplifier \((50+0i)\Omega\), and the power coupled into the plasma is higher. As expected, a similar behaviour is seen in the case of the pulsed plate electrode configuration (figure 3.15(b)).

Figure 3.15(c) shows the waveforms for the ring electrode configuration. Comparing the signals of the three free plasmas, the lowest phase shift and therefore best matching is obtained for the ring electrode configuration. In this case the phase shift stays nearly constant. Due to the configuration and the strong discharge between needle and ring, the load is well defined and does not change significantly with plasma conditions in the DBD-like structure. The power is also increasing, which is not mainly due to the phase shift as in the two previous cases but due to the change of voltage and current amplitude. Contrary to the plate electrode cases this results in a higher apparent power in this case and a larger power coupling to the plasma.

Comparing the power dissipation in the touching and non-touching mode for the three investigated conditions, the plate electrode shows the biggest change. The ring electrode shows the smallest relative change as also seen in the previous section.

When the plasma is in contact with a conductive substrate, we assume that the main change is a drop in plasma resistance, due to a higher electron density and an increase in sheath capacity, due to a breakdown in the plasma sheath. This is also seen in glow discharges that experience a mode transition from the \( \alpha \)– to the \( \gamma \)–mode (see for atmospheric pressure e.g. [34, 35]). This leads to a favourable matching resulting in more power coupled into the load, which in turn leads to a higher dissipated power and a reduction in phase shift. This effect is less dominant for the ring electrode since the majority of the active plasma is created in between the electrodes, and is less affected by the surroundings.

3.5 Spatially resolved gas temperature

A change in power dissipation leads to a variety of changes in several plasma parameters. As the heat flux to a treated substrate is a very important parameter for biological applications, the spatially resolved gas temperature of the three different cases presented in figure 3.15 is shown in figure 3.16.

The gas temperature is obtained by the rotational emission band of OH(A-X)(0,0). Figure 3.16 shows either the gas temperature without any substrate or the gas
3.5: Spatially resolved gas temperature

Figure 3.16: Spatially resolved gas temperature in axial direction as obtained from the rotational emission band of the OH(A-X)(0,0) transition. The experimental conditions are the same as in figure 3.15.
temperature when the plasma is in contact with water substrate at a distance of approximately 7 – 9 mm, depending on the configuration. The same input parameters for the RF amplifier have been used as in the previous section.

Figure 3.16(a) shows the spatially resolved rotational temperature for the continuous RF driven plate electrode configuration, starting from the needle electrode position on the left side of the graph until the position at which the plasma is touching the water substrate on the right side of the graph. When the plasma touches the water, the gas temperature is consistently higher, compared to the gas temperature of the free plasma. In the latter case the gas temperature inside the glass tube is around 380 K and rises after it leaves the glass tube to around 440 K before it reaches a constant value at 380 K. The increase of the gas temperature once the plasma leaves the glass electrode can be explained by the admixture of air. By adding molecules, more chemical reactions occur which transform excess energy from the plasma into the heating of the gas increasing the thermal output. A less pronounced effect of this has also been observed by Zhang et al. [37]. For the touching plasma, the gas temperature is around 50 K higher than in the case of the free plasma. This is to be expected since the plasma power is also higher.

The advantage of the time modulated plasma (shown in figure 3.16(b)) becomes clear from the gas temperature in the case of the undisturbed plasma. Although the instantaneous power is higher, the average power is reduced due to the modulation, leading to a lower gas temperature compared to the continuously driven grounded plate electrode configuration. However, the advantage of modulation is changed the moment the plasma is in direct contact with the water substrate. The gas temperature increases by approximately 80 K in this case, from 350 to 430 K. The increase of the gas temperature and power is similar as in the former case, although the gas temperature difference is getting larger closer to the water substrate. This is consistent with the fact that the plasma is mostly influenced by the water substrate between the plate electrode and the water.

Figure 3.16(c) shows the spatially resolved gas temperature for the grounded ring electrode configuration. For both conditions a maximum of the gas temperature is found between 4 and 5 mm behind the glass tube and the ring electrode in the order of 440 K and 460 K for the undisturbed and the touching plasma, respectively. Afterwards, the gas temperature drops down to 340 K at the end of the plasma plume. The power and average gas temperature are higher than for the modulated plate electrode configuration, indicating again that the discharge is the strongest inside the glass tube and only a small fraction is created behind the grounded ring electrode. A higher gas temperature when the discharge is in contact with the water substrate is also observed in this configuration, although the increase is smaller. The temperature drops after 5 mm to the same level as with the free plasma.
3.6: Different materials in contact with the ring electrode configuration

In this section further examples for the ring electrode configuration are shown in contact with a dielectric material (glass) and metal. Images of the two conditions are shown in figure 3.17. These images illustrate, that the plasma does not change significantly when in contact with a dielectric. In contact with metal, the diffuse part of the discharge disappears completely and a plasma filament, similar as seen in the modulated plate electrode geometry, is observed. This indicates that, when the distance between a conductive substrate and the needle electrode is close enough, the ring electrode does not play a significant role as electrode any more.

Figure 3.18 and figure 3.19 show time resolved ICCD images and the corresponding time resolved power, for the plasma in contact with glass, metal and the free plasma. The images of the free plasma are shown in figure 3.13(a). As expected, the plasma touching the glass plate does not show any significant difference in power, compared to the free plasma. The plasma touching the metal substrate shows similar behaviour as the plasma in contact with the water substrate, i.e. a higher power and higher emission, when in contact with the water (at 5 \(\mu\)s).

The electrical characteristics during one RF pulse for the three conditions are shown in figure 3.20. Figure 3.21 shows the spatially resolved gas temperature, corresponding to the electrical characteristics. As can be expected from the images, no significant change in electrical characteristics or in the gas temperature is observed when the plasma is in contact with a glass substrate, compared to the free plasma. If metal is used as substrate, power and gas temperature are increasing (with a maximum gas temperature at approximately 1 mm in front of

![Figure 3.17: CCD images of the plasma with the ring electrode configuration in contact with a glass (a) and a metal substrate (b).](image-url)
Figure 3.18: ICCD images of the plasma with the ring electrode configuration in contact with a glass (a) and a metal substrate (b) at different time points of the pulse modulation as indicated in the images. The dotted lines indicate the end of the ring electrode and the position of the glass and metal substrate, respectively. The corresponding power is shown in figure 3.19.

Figure 3.19: Time resolved power (averaged over one RF cycle) for a free plasma and a plasma touching a glass and metal substrate. The experimental conditions are the same as in figure 3.18.
3.7: Conclusion

In this chapter measurements of the plasma dissipated power (time resolved) and the gas temperature (spatially resolved) of an atmospheric pressure RF driven plasma jet operated with three different configurations, while in contact with various substrates of different conductivity and permittivity (water, glass, metal) are presented. It is shown that, when a conductive substrate is too close to the plasma plume, the power dissipation in the plasma and the gas temperature increase. Time resolved measurements of the plasma morphology and the power indicated that the increase of power is only starting the moment the plasma touches the conductive substrate. An increase of up to a factor of 3 in power has been observed for the plate electrode configuration, while a factor less then 1.5 is observed for the ring electrode configuration. The change in impedance of the plasma source, leading to a more favourable matching, is shown to be the main reason of the observed change in electrical characteristics. In addition, when in direct contact with a conductive substrate, the gas temperature of the plasma jet increases in all observed cases with a maximum of up to 80 K.

The electrical properties of the material, which is treated in direct contact with the plasma, strongly influences the plasma (parameters) and diagnostics need to
be applied for identical conditions. This also indicates, that when conductive meshes are used, as for example to reduce the charge flux onto the substrate in biological experiments, it is likely that the plasma is changing (e.g. increase of the density of charged and reactive species), resulting in an increased flux of reactive species.
References


Chapter 4

In situ absolute air, O₃, and NO densities in the effluent of an RF argon APPJ obtained by molecular beam mass spectrometry *

Abstract

In this chapter a molecular beam mass spectrometer has been calibrated and used to measure the air entrainment, nitric oxide and ozone concentrations in the effluent of a cold atmospheric pressure argon RF plasma jet. The approaches for calibrating the mass spectrometer for different species is described in detail. Gas phase concentrations of ozone and nitric oxide up to 7.5 and 4 ppm, respectively, have been measured in the far effluent of the plasma jet argon plasma jet. The difference in air entrainment when the plasma is undisturbed or close to a well, which is the case e.g. for in vitro plasma-cell interaction studies, is shown. In addition an exponential decay of the positive ion flux as a function of distance in the effluent is obtained. Furthermore the effect of plasma power, duty cycle and air and O₂ admixtures introduced into the argon flow on the NO and O₃ production is presented including the possibility of independent control of the NO and O₃ flux from the jet.

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4.1 Introduction

Cold atmospheric pressure plasma jets (CAPPJs) produce large fluxes of reactive species, such as ozone [1], nitric oxide [2] or hydroxyl [3], which are known to be important for biological processes (see e.g. [4–6] and references therein). The amount of e.g NO is very critical for biological systems. Small amounts of NO could stimulate wound healing while large amounts will induce cell death [4]. It is therefore very important to know the amount of reactive species which reach the biological sample. A review summarising the key aspects of CAPPJs can be found in [7].

The goal of this chapter is to investigate the gas phase density of stable chemical compounds (O$_3$, NO$_x$), the flux of ions from the plasma jet and the air mixing in the effluent at the position of a substrate. Mass spectrometry is used in this study and the results are compared with laser induced fluorescence (LIF) measurements (NO density) and adsorption spectroscopy measurements (O$_3$ density) performed on the same plasma jet [1, 2]. LIF is a very selective diagnostic method which requires accurate gas temperature measurements and often a complex calibration (see e.g. Verreycken et al. [8]). In addition absorption spectroscopy requires to perform an Abel inversion to obtain spatial resolved densities which is difficult to perform for small sized (not perfectly cylindrical symmetric) plasmas.

In contrast, mass spectrometry can detect a large variety of species created by a plasma, ranging from ions, metastables and reactive species (see e.g. [9] and references therein). The absolute calibration however needs to be obtained by performing a reference measurement of a known concentration of the species of interest. The main advantage is, that by mass spectrometry species densities at the substrate can be obtained directly at the surface where plasma-effluent interaction happens, if a small orifice can be embedded into the substrate.

In this work we present absolutely calibrated densities of nitric oxide and ozone in the effluent of a radio frequency argon plasma jet in air measured by molecular beam mass spectrometry (MBMS).

This chapter is structured as followed. First, we describe the experimental setup in section 4.2. Next, the measurement procedure and the absolute calibration methods are introduced in section 4.4. Finally, in section 4.4 the obtained ion fluxes, air concentration, nitric oxide, and ozone densities in the plasma effluent are presented. In particular, the more realistic situation in case of a presence of a well plate structure will be discussed.
4.2 Experimental setup

The plasma source is a CAPPJ consisting of a plastic enclosure containing a concentric powered needle electrode with a diameter of 1 mm surrounded by a glass tube with an inner diameter of 1.65 mm and a 5 mm long grounded ring electrode positioned about 1 mm away from the tube edge. The needle is connected to a matching circuit which is connected to a radio frequency power amplifier (Amplifier Research 75 W, 5-250 MHz) driven at 13.56 MHz by a function generator (Agilent 33220A). A second function generator of the same type can be used to apply a kHz modulation of the RF signal. The generator producing the RF signal is set to frequency-shift keying (FSK) (switching frequency between 13.56 MHz and 1 µHz) and is triggered by the second function generator by means of a TTL square waveform at 20 kHz. The plasma is generated by flowing argon (purity 5.0, 1.5 slm) with variable admixtures of air or oxygen. The gas flows are controlled by two mass flow controllers (Brooks 5850E).
The RF (13.56 MHz) voltage is applied to the needle electrode. The plasma power is obtained as described in chapter 2. Figure 4.1 shows a schematic of the setup used for the measurements obtained with the MBMS. The plasma is mounted on an x,y,z-stage, adjustable with micrometer screws (not shown). The nozzle of the plasma jet is placed above the metal orifice with a diameter of 50 µm which is used for the gas sampling to the MBMS. As the orifice is in a metal plate, all measurements presented here have been performed in a non-touching condition in order not to disturb the plasma (see chapter 3).

To approach the flow conditions which are present during the treatment of microorganisms in solutions, a part of a 96-well plate with the bottom removed is placed on the sampling plate containing the orifice. As the sampling orifice is mounted on a ring with screw threads which enables the variation of the pinhole position, the CAPPJ and its effluent expands into a cup-like structure. In order to prevent the accumulation of gases in this volume, the gas is removed by a "suction-ring". The suction-ring is a tube with an outer diameter of 80 mm, containing small holes, and is connected to a rotameter and a vacuum pump (at a flow rate of 30 slm) to avoid that the concentration of argon inside the cup increases as a function of time. This is important as the change in air concentration, surrounding the plasma jet, could change the plasma chemistry. The well and the suction ring are always installed during the measurements, if not indicated otherwise.

The mass spectrometer is a Hiden EPIC/IDP (Series 1000). It has a Bessel box energy analyser to block UV photons and electrons from reaching the detector. For the calibration measurements of air a vacuum chamber is attached to the MBMS system (indicated in red dashed lines in figure 4.1).

The mass spectrometer operates at pressures below $6 \cdot 10^{-9}$ bar. The plasma jet however, operates at atmospheric pressure. For this reason the plasma is sampled through a 50 µm metal orifice, which is connected to a three stage pressure reduction system. The pressure reduction system is continuously pumped down by turbo pumps (Pfeiffer HiPace 300) and pre-pumps (Boc Edwards XDS 10). The pressure in the first stage (in the order of $10^{-6}$ bar) is measured using a Pirani pressure gauge (Edwards APG-L-NW 16). The pressure in the second and the third stage is approximately $10^{-9}$ bar and $10^{-10}$ bar, respectively and is measured by cold cathode gauges (Edwards AIM-S-NW 25, Edwards AIM-X-M/SEAL DN40CF, respectively).

Due to the small orifice and the fast pumping rate in the first stage, the pressure is low enough so that the mean free path in the first stage is larger than the distance to the first skimmer. In this case a Mach disk is not formed in the first stage and the supersonic gas flow entering the first stage follows a smooth transition from a collisional dominant region to a molecular flow region [10]. The cone-shaped skimmer is placed inside this molecular flow region, approximately 5 mm downstream of the orifice.
4.3 Calibration

In order to obtain absolute densities of the air, nitric oxide and ozone reaching the treatment surface, the mass spectrometer is calibrated. In this section the calibration methods are described in detail.

4.3.1 Air

For the calibration of air, a vacuum chamber is attached on top of the MBMS as indicated in figure 4.1. The mass spectrometer is absolutely calibrated for the air signal by filling the vacuum chamber on top of the MBMS with different argon/dry-air mixtures. After the chamber has been filled, the percentage of air is deduced from the mass spectrometry measurements by introducing $S_{\text{ratio}}$:

$$S_{\text{ratio}}[\%] = \frac{S_{N_2} + S_{O_2}}{S_{N_2} + S_{O_2} + S_{Ar}} \cdot 100\%,$$  \hfill (4.1)

with $S_X$ being the detector signals (given in counts/sec) measured for argon (40 amu/z), $O_2$ (32 amu/z) and $N_2$ (28 amu/z). $S_{\text{ratio}}$ is compared with the percentage of air introduced into the chamber. The result of this calibration measurement is shown in figure 4.2. A linear relation between $S_{\text{ratio}}$ and the air concentration introduced by the mass flow as expected is observed. The discrepancy between these two signals is about 4%.

![Air calibration curve obtained with a vacuum chamber attached to the MBMS.](image)

Figure 4.2: Air calibration curve obtained with a vacuum chamber attached to the MBMS.
4.3.2 Nitric oxide

Figure 4.3: Example of the raw data obtained at 30 amu/z during a NO calibration. The jump in signal is due to the change of various concentrations of premixed NO into the argon flow. A fit of the background signal is shown in red. The amount of NO added to the argon flow is indicated in the figure. No well plate is present.

To calibrate the MBMS for NO, a gas flow with known NO-concentration is flushed through the plasma jet (plasma switched off). The gas mixture is prepared by mixing argon (purity 5.0) with a test-gas mixture containing (203 ± 1) ppm NO in argon (Linde gas) with two mass flow controllers. During the calibration measurements, the jet is placed at a distance of approximately 3 mm between the orifice and nozzle. At this distance the amount of surrounding air mixed into the argon effluent is negligible which can be seen from the measured lines of O\textsubscript{2} (16 and 32 amu/z) and N\textsubscript{2} (14 and 28 amu/z) (data not shown).

The background of the signal at 30 amu/z is relatively large, resulting, among other things, from impurities of the vacuum system and the MS chamber, air entrainment (e.g. N\textsubscript{2} isotopes and other residual gases). However, a change of background occurs on time scales of several minutes while, any change of the beam components changes within a millisecond [11]. This difference in time constant is used to minimise the influence of the changing background. The calibration is performed by switching from an argon to an argon-NO gas flow while continuously measuring the MBMS signal at 30 amu/z. The difference in signal measured between these two stages is correlated with the known NO density.

An example of a calibration measurement is shown in figure 4.3. At $t = 0$ min a pure argon flow is applied. After 29 minutes the flow is switched from a pure argon flow to an argon flow with $3.4 \cdot 10^{20}$ m\textsuperscript{-3} (13.6 ppm) NO. It has to be noted...
4.3: Calibration

that in this graph the initial increase in signal after 30 minutes is rather slow. This is due to the fact that the gas tubes still had residual air inside at the first measurement. Furthermore, less impurities from the vacuum and MBMS devices may be released after a certain time of operation. At $t = 50$ minutes the flow is switched back to a pure argon flow. As outlined above, the sudden drop and increase in signal is used to separate between the beam and the background signal. Note that the trend of the background is not significantly disturbed by the fact that the flow is switched from argon to argon-NO, during the measurement campaign.

If the distance between the plasma source and the orifice is varied, the main components will change from pure argon to argon with air, due to an increase in air concentration in the effluent of the jet at increasing distances from the nozzle. In order to investigate the change of the main components from argon to air, the NO calibration has been performed with argon-NO mixed with different concentrations of argon and dry air. No significant effect of the measured NO density by varying the argon and air ratio is observed (data not shown).

Figure 4.4: NO and $O_3$ calibration curve for the experimental conditions and settings for which the measurements are performed. No well plate is present.

Figure 4.4 shows the results of the nitric oxide calibration for our MBMS system. A linear fit gives a calibration factor of $(7.36 \pm 0.10) \cdot 10^{16}$ m$^{-3}$/counts/s for nitric oxide. The error bars in the measured NO concentration reveal the fluctuation in the measurements and the accuracy of the used mass flow controllers. The detection limit of NO under the calibration conditions is 0.1 ppm. This is mostly due to the changing background during the NO measurements.
4.3.3 Ozone

To calibrate the MBMS for ozone the MBMS measurement is compared with an absorption measurement at the same setup. This was implemented as follows: various concentrations of ozone were produced by the plasma jet, which was operated outside the MBMS ring in a closed, gas tight chamber, by varying the plasma power and the oxygen concentration in the gas flow. The effluent containing ozone was then guided through silicone tubes into the absorption chamber. In this chamber the O\textsubscript{3} density was obtained by UV-absorption, as described in [1]. The gas from the chamber was further guided to the mass spectrometer in a short silicone tube and the signal at 48 amu/z is compared with the corresponding absorption measurement.

Next to the calibration curve for nitric oxide, figure 4.4 shows the results of the ozone calibration. A linear fit gives a calibration factor of \((4.39 \pm 0.12) \cdot 10^{17} \text{m}^{-3}/\text{counts/s}\) for ozone. As for the nitric oxide measurements, the error bars in the measured NO concentration contain the fluctuation in the measurements and the accuracy of the used mass flow controllers. The detection limit of O\textsubscript{3} is 1 ppm. This is mostly due to fluctuations in the measurement signals for O\textsubscript{3}.

4.4 Results

4.4.1 Ions

The MBMS can also be used to measure positive ion fluxes produced by the plasma jet. In this case the first and second skimmer were biased to \(-10\) V and to \(-40\) V, respectively. Due to the high sensitivity of the system the relative signal of positive ions as a function of distance from the glass nozzle could be obtained up to 7 mm away from the plasma tip. The total positive ion counts are shown in figure 4.5.

Positive ion signals are found at amu/z-values corresponding to H\textsubscript{3}O\textsuperscript{+} and its water clusters. H\textsubscript{3}O\textsuperscript{+} and higher protonated water clusters have been observed as the dominant ions in corona discharges in air (see e.g. [12]) and RF plasma jets in He-O\textsubscript{2} gas mixtures [13]. The other dominant signal at 18 amu/z is unlikely to be H\textsubscript{3}O\textsuperscript{+} as these ions reacts efficiently with water molecules forming the H\textsubscript{3}O\textsuperscript{+} [13]. Another possibility is the ammonium ion NH\textsubscript{4}\textsuperscript{+} as argued in [14].

As the electron density outside of the ring electrode is of the order of a few \(10^{19} \text{m}^{-3}\) [15] it is clear that outside the emission zone of the plasma the density of ions is much smaller than the reactive species densities, such as NO [2].
4.4: Results

Figure 4.5: Positive ion counts as a function of the distance from the nozzle to the metal orifice at 1.1 W continuous plasma power and a 1.5 slm argon flow. The MBMS is equipped with a suction ring with a flow rate of 30 slm, without a well attached to the sampling plate. The length of the visible plasma plume is approximately 3 mm. A spectrum is shown at a distance of 5 mm between the nozzle and the orifice.

4.4.2 Air

Figure 4.6 shows the measured air concentration as a function of distance between nozzle and metal orifice. The flow applied through the plasma jet is 1.5 slm. The measurements are performed with an argon flow and the plasma switched off with and without placing the well on the orifice of the MBMS. In both cases, close to the orifice, no air entrainment of surrounding air is observed. At a distance of 30 mm between the electrode and the orifice of the MBMS, the air entrainment increases up to an air concentration of 50 to 60%. The increase of the percentage of air is lower when the well is attached compared to the case when the well is not attached to the metal sampling plate.

The lower air concentration with the well attached to the sampling plate can be explained by the local enrichment of argon due to the flow pattern as schematically sketched in figure 4.7. If the nozzle is further away, a significant amount of air will be mixed into the flow, when it reaches the well. These results show the need of the well mounted on the MBMS to simulate similar flow conditions as present during the treatment of biological material in solutions in well plates. Because argon fills the area around the sampling orifice of the mass spectrometer, the effect of the suction ring is determined by changing the flow through the suction ring and measuring the change of air concentration with and without the mounted well.
Figure 4.6: Air concentration as a function of the axial distance, with and without a well attached. A flow of 1.5 slm of argon is applied through the plasma jet as well as a 30 slm flow rate through the suction ring.

Figure 4.8 shows the percentage of the air entrainment as a function of the pumping volume. If the suction is not used (0 slm), the cup surrounding the orifice is filled with argon after some time, as argon is heavier than air. When the pumping volume is increasing, more argon is removed from the cup and more ambient air is mixed into the volume of the cup and the effluent of the jet. The value of the air concentration at 20 mm away from the nozzle of the jet approaches a constant value of approximately 30%. The value "max" in the pumping flow rate shown in figure 4.8, with the vacuum pump directly attached to the suction ring, is much larger than 30 slm and exceeds the read out of the rotameter. Since the air concentration is nearly constant starting from a suction flow of 20 slm a value of 30 slm has been used.

In accordance with van Gessel et al. [15] a change in air densities in the effluent between the plasma switched on and off has been found. Nonetheless the presence of a well significantly complicates the flow pattern and a detailed study on the effect of the plasma on the air entrainment for this configuration is outside the scope of this work.

4.4.3 Nitric oxide

Figure 4.9 shows the absolute NO density as a function of the average plasma power and duty cycle. It can be seen that the NO density is proportional to
4.4: Results

Figure 4.7: Sketch of the flow pattern and air entrainment with and without well to schematically illustrate the local argon enrichment in the presence of a well.

In order to validate the MBMS measurements of nitric oxide, the results are compared with LIF measurements of NO performed which are reported in [2] for the same plasma jet (see figure 4.10). The NO density decreases with increasing distance in the effluent due to outwards diffusion and reactions such as,

\[
\text{NO} + \text{O}_3 \rightarrow \text{NO}_2 + \text{O}_2.
\]  

(4.2)

Comparing the results of the mass spectrometer measurements with the LIF measurements, it is found that the NO density obtained by MBMS is systematically larger compared to the results of the LIF measurements. The discrepancy close to the nozzle is 25% and reaches a factor 4 in the far effluent. This offset could be due to the presence of NO₂, N₂O₅, HNO₂, HNO₃ of which cracking patterns overlap with the MS-signal at 30 amu/z. Unfortunately, not all cracking patterns of these species are published. However, the contribution of NO₂ was checked by measuring simultaneously the signal at 30 amu/z and 46 amu/z. When correcting for the cracking pattern [17] it is found that the contribution of NO₂ to the NO
Figure 4.8: Air concentration as a function of the flow rate through the suction ring with and without a well attached. The distance between the plasma jet nozzle and the orifice is 20 mm. A flow rate of 1.5 slm is applied through the plasma jet.

signal is less than 10% for the present experimental conditions. Another difference which contributes to the slight discrepancies of these two methods is the fact that the LIF measurements have been performed with the plasma jet pointing opposite to the direction of gravity while the MBMS measurements have been obtained with the plasma pointing in the direction of gravity close to a sampling orifice. This discrepancy will affect the flow pattern and air entrainment.

Note that the NO density is roughly constant with increasing distance when no air is added into the argon flow (see figure 4.10). This suggests that the air entrainment in the afterglow has a minor effect on the NO density. The graph with no air added is showing results obtained with the well attached to the metal orifice. However, the NO densities when sampled with or without the well attached to the orifice is found to be the same within the experimental accuracy.

Figure 4.11 shows the effect of premixing air into the argon flow on the NO concentration. For these measurements, the distance between plasma jet and metal orifice has been kept fixed at 17 mm. Even when the power is kept constant, adding air to the flow shortens the visible plasma length as the plasma is quenched in the effluent by the air. It can be seen that a larger air concentration at constant power, while keeping the plasma power constant, results in a higher NO density, as expected and observed in [2]. In [2] a maximum of NO density has been found when 2% of air are mixed to the argon flow, while the mass spectrometer results show a steady increase of NO with increasing the air admixture. The LIF measurements in [2] have been performed at a distance of 5 mm from the
4.4: Results

Figure 4.9: NO density as a function of the average plasma dissipated power at different duty cycles with a 1.5 slm argon flow. The MBMS entrance is equipped with the suction ring and well. The distance between visible plasma tip and metal orifice has been kept constant at 8 mm. nozzle, while the MBMS measurements are performed at 17 mm. This could cause contributions due to HNO₃, HNO₂ or N₂O₅ in the MBMS measurements which are not included in the LIF measurements. When the same percentage of oxygen is added into the argon flow, the NO density is below the detection limit of the MBMS.

4.4.4 Ozone

Figure 4.12 shows the ozone density as a function of the average power for different duty cycles. While the NO density is linearly increasing with the average power and is independent of the duty cycle (see figure 4.9), the ozone density quadratically increases with the average power and decreases with increasing duty cycle.

The production and destruction rates of ozone in the same plasma jet studied in this work have been investigated by Zhang et al. [1]. The main production reaction for the formation of ozone is the three-body reaction of O with O₂:

\[ O + O₂ + M \rightarrow O₃ + M. \]  (4.3)

With increasing power the O density will increase and as such the production in the far effluent of O₃ is also expected to increase. The reduction of the duty cycle
Chapter 4: NO, O₃ and air densities in the argon effluent of an RF CAPPJ

Figure 4.10: Comparison of the NO density measured by LIF and MBMS, as function of distance between the nozzle of the jet and the metal orifice for a 3.5 W plasma, 20 % duty cycle, 1 slm argon, premixed with 2 % air. The LIF data is taken from [2], with no sampling plate present in front of the jet. The MBMS is equipped with the suction ring with 30 slm flow and no well attached to the orifice. As comparison, the NO density as function of the distance for a 3.6 W plasma, 1.5 slm argon flow without air added to the gas flow and the MBMS equipped with a well is also shown.

at a constant average power leads to an increase in instantaneous power, and thus to higher peak O densities. In addition, during the plasma off time most of the atomic oxygen is recombining to O₂ and O₃. The decrease in duty cycle leads to similar trends in ozone density as compared to increasing the average power.

The dependence of the ozone density on the distance from the nozzle is shown in figure 4.13 for argon, argon with 2 % O₂ and argon with 2 % air. The O₃ concentration increases with increasing distance in the far effluent of the plasma jet. At distances larger than 20 mm the O₃ concentration remains constant at its maximum value for the pure argon case, while this is in the range between 12 and 15 mm in the other cases.

The increase in the ozone density with increasing axial distance coincides with the drop of the O density as is shown in [1]. As the gas temperature is rather close to room temperature in the far effluent, the lifetime of O₃ is relatively large outside the active plasma which leads to a constant ozone density for distances up to 30 mm from the jet. The decrease is partly due to radial diffusion and widening of the ozone radial density profiles with increasing axial distance [1].

The effect on the ozone density as a function of the mixing of air and oxygen to
the argon flow at a distance of 15 mm between the nozzle and the metal orifice is shown in figure 4.14. A larger admixture of both air and O\textsubscript{2} results in a higher O\textsubscript{3} production.

Premixing O\textsubscript{2} into the argon flow increases the ozone density significantly more compared to mixing the argon flow with air as more O\textsubscript{2} is available for the production of O and O\textsubscript{3}. Indeed, the O\textsubscript{3} density for 0.4 % O\textsubscript{2} is only slightly higher than the 2 % air case (which contains approximately 0.4 % oxygen). Additional loss mechanisms in air, due to oxidation of NO, could explain the small differences.

\textbf{Figure 4.11}: NO density for different admixtures of air into a 1.5 slm argon plasma with 2.6 W continuous power. The distance between the nozzle and orifice is kept constant at 17 mm.
Figure 4.12: $O_3$ density as a function of the average plasma dissipated power at different duty cycles with a 1.5 slm argon flow. The MBMS entrance is equipped with the suction ring and well. The distance between visible plasma tip and metal orifice has been kept constant at 8 mm.

Figure 4.13: $O_3$ density as a function of the distance between the nozzle of the jet and the metal orifice for an argon plasma with and without admixtures of air and oxygen. The visible plasma tip is at 8 mm distance from the orifice for the first measurement point. The plasma power is in all cases 1 W, the duty cycle is 20%, the flow through the jet is 1.5 slm.
4.5 Conclusion

In this work we measured the air concentration, NO, O$_3$ density and ionic species in the effluent of a cold atmospheric pressure argon plasma jet by a molecular beam mass spectrometer. The MBMS is absolutely calibrated for nitric oxide, ozone and air. The ions show an exponential decay leading to densities which are at least 2 orders of magnitude smaller compared to the NO and O$_3$ densities. It is shown that the presence of a well as in the case during in vitro plasma treatment of biological solutions, has a significant effect on the air concentration in the jet effluent. NO densities in the range of 0.8 to 4 ppm and ozone densities in the range of 1 to 7.5 ppm are measured for an argon plasma without admixtures of air or oxygen. By mixing oxygen into the argon flow ozone densities up to 100 ppm and with adding air, nitric oxide densities up to 30 ppm are detected in the jet effluent, for the investigated experimental conditions. Both the NO and the O$_3$ density increase with increasing power and air concentration mixed to the argon flow. Switching between O$_2$, air mixtures, plasma power and duty cycle allows to increase or decrease the nitric oxide and ozone densities independently.

Figure 4.14: O$_3$ density for different admixtures of air and O$_2$ added to the 1.5 slm argon flow. The jet operated a 2.6 W plasma dissipated power and 20% duty cycle. The distance between the nozzle and orifice is kept constant at 15 mm.
Chapter 4: NO, O₃ and air densities in the argon effluent of an RF CAPPJ
References


Chapter 5

Mechanisms of bacterial inactivation in the liquid phase induced by a RF CAPPJ *

Abstract

A radio frequency atmospheric pressure argon plasma jet is used for the inactivation of bacteria (Pseudomonas aeruginosa) in solutions. The source is characterized by measurements of power dissipation, gas temperature, absolute UV irradiance as well as mass spectrometry measurements of ions, NO and O₃. The plasma induced liquid chemistry is studied by performing liquid ion chromatography and hydrogen peroxide concentration measurements on treated distilled water samples. Additionally, a quantitative estimation of an extensive liquid chemistry induced by the plasma is made by solution kinetics calculations. The role of the different active components of the plasma is evaluated based on either measurements, as mentioned above, or estimations based on published data of measurements of those components. It is shown that HNO₂, ONOO⁻ and H₂O₂ are present in the liquid phase in similar quantities as concentrations which are reported in literature to cause bacterial inactivation. In addition a threshold effect of bacteria inactivation as function of the distance between plasma and liquid is reported which indicates the importance of synergistic effects between H₂O₂ and acidified NO₂⁻.

5.1 Introduction

In this chapter an in vitro study of the bactericidal effect is presented in distilled water and the agents responsible for this effect are identified. The plasma source is characterized using measurements of power dissipation, gas temperature, absolute UV irradiance as well as mass spectrometry measurements of ions, NO and O$_3$ fluxes. Results of the treatments of the bacterium P. aeruginosa are shown. An analysis is made of the various active plasma components and their contribution to the observed biological effects is quantified.

The chapter is outlined as follows. First the experimental setup is introduced, after which examples of bacterial inactivation are shown. The effects of the individual components of the plasma are discussed and their influence on the bactericidal properties of the plasma is assessed. Finally a rough description of the induced fluid chemistry is provided by combining measurements of treated liquids with solution kinetics simulations. In addition a threshold inactivation is found for a critical distance of the plasma jet to the water from which (indirectly) synergistic effects between H$_2$O$_2$ and acidified NO$_x$ are deduced.

5.2 Experimental setup and methods

5.2.1 Plasma source

The plasma jet and the power source are the same as used in the previous chapters. A schematic of the setup is shown in figure 5.1. The needle is connected via a matching circuit to a radio frequency power amplifier (Amplifier Research 75W, 5-250 MHz) driven at 13.56 MHz by a wave generator (Agilent 33220A). A second wave generator of the same type was used to apply a kHz modulation to the RF signal. The generator responsible for the RF signal is set to frequency-shift keying (FSK) (switching frequency between 13.56 MHz and 1 µHz) and is triggered by the second function generator by means of TTL pulses at 20 kHz. The measurements presented in section 5.4 are obtained with a continuously driven plasma.

To reduce the amount of reflected power a matching circuit is connected in series between the power amplifier and the plasma source. This home made matching circuit consists only of a coil. This rudimentary electric circuit design (compared to an L-section or Π-section) minimizes the amount of stray impedances and enables easy and accurate measurements of the power dissipation. As a result the entire current passes through the coil and the plasma source.

The gas flow is controlled by a mass flow controller (Brooks 5850E) and argon with a purity of 99.999% is used as a carrier gas. The treatments and diagnostics presented in this work are all performed in distilled water. A gas flow of 1.5
5.2: Experimental setup and methods

Figure 5.1: Schematic representation of the plasma source equipped with a ring electrode showing the power supply, voltage and current probes, matching circuit, plasma source. The part of the plasma plume, visible to the naked eye, has a length of approximately 6 mm. The dimensions are not to scale and note that the inner diameter of the glass tube of the plasma jet is 1.65 mm and the inner diameter of the well is 6.6 mm.

slm of argon, a duty cycle of 20% (i.e. RF applied for 10 µs every 50 µs) and a time-averaged plasma dissipated power of (1.4 ± 0.1) W is used.

5.2.2 Plasma diagnostics

The average power dissipation is measured by measuring the voltage (1:100 Tektronix P5100 voltage probe) and current (Pearson 2877 current probe) and correcting for the time delay between the probes. The probes are read out by an oscilloscope (Agilent Technologies DSO 1024A, 200 MHz, and 2 GSa/s). The power dissipation in the matching circuit is corrected as described in chapter 2, to obtain the plasma dissipated power.

The measurements of the gas temperature inside the plasma are performed using Boltzmann plots of the OH (A-X) transition at 309 nm as described in chapter 2. In the current study however a two lens system using UV-lenses is used to project an image of the plasma on the entrance slit of the spectrometer yielding a spatially resolved spectrum with a resolution of 0.2 mm. The gas temperature in the effluent is measured by using a type K thermocouple (Fluke 80BK-A), at distances where there is no direct contact with the plasma jet.

The absolutely calibrated spectrum is measured by using an spectrometer (Avantes AvaSpec 3648) in combination with a 1 mm orifice and a lens, coaxially aligned with the plasma. A schematic of this setup is shown in figure 5.2. The spectrom-
The measurements of the concentration of nitrite and nitrate have been performed by ion-chromatography of distilled water treated with the plasma jet. For these measurements a minimum volume of around 1 ml is necessary. Well plates containing 12 wells with a maximum volume of 6.5 ml have been chosen to perform the treatment and a volume of 3.35 ml is treated to allow for multiple ion chromatography runs.

The system used for the measurements is an ion chromatography system (*Dionex ICS-90*) in combination with an auto-sampler. The eluent is a mixture of sodium carbonate and sodium hydrogen carbonate and the separation column is made of packed resin. After exiting the column a suppressor is used to minimize the background conductivity of the eluent and thus enhancing the detection of sample ions. The conductivity of the resulting solution is measured using a thermally controlled digital conductivity meter, allowing a detection range of up to $10^5 \mu S/cm$. Because of the sensitivity of the chromatography setup only distilled water can be used. In order to correct for increased concentrations due to evaporation of the water as a result of plasma treatment, the evaporation is determined by measuring the mass of the well plate including water before and after treatment.

Hydrogen peroxide ($H_2O_2$) concentration in distilled water after plasma treatment is determined by measuring the absorption of light emitted by a blue LED at 450 nm by a mixture of plasma treated water and an ammonium metavanadate solution at a ratio of 1:1. The reaction of $H_2O_2$ and metavanadate in an acidic medium results in the formation of peroxovanadium cations which produce a red-orange colour with a maximum absorbence at 450 nm [1]. The advantage of using ammonium metavanadate is that it is sensitive to hydrogen peroxide in the liquid but shows little to no effect from concentrations of nitrate, chloride or ferric iron [1]. Further, work of Dodet *et al.* [2] indicates that the ammonium metavanadate detection is not significantly influenced by ozone.
By comparing the absorption to a reference mixture of non-treated distilled water and metavanadate solution, the concentration of H$_2$O$_2$ can be calculated. The treatment volume is 3.8 ml and we also took evaporation into account as described above.

As the biological treatments are performed in a smaller volume (100 µl), see further, correction for the bigger volume is made assuming that the plasma induced effects scale with the volume of the treated liquid, i.e. that the similar effects are expected for the same treatment time per volume (or proportional treatment time). For example, a treatment time of 1 minute on a volume of 100 µl and a treatment time of 30 minutes for a sample volume of 3 ml result in comparable concentrations of reactive species. This assumption is only valid when the solution is well mixed. Experiments obtained with a similar plasma jet and a similar flow, as assumed in this work, showed that the gas flow induced mixing in the liquid is of the order of 10 mm/s [3], which means that the mixing is orders of magnitude faster than the diffusion of reactive species as observed e.g. in the work of Oehmigen et al. [4], which guarantees good mixing on the time scales of minutes. Further motivation on the proportional treatment time approximation is presented in the sections (5.3.1 and 5.3.3).

5.2.4 Bacteria treatment

In this work Pseudomonas aeruginosa (PAO1) is used for the inactivation study. This is a Gram-negative bacterium, which can cause diseases and survives on most surfaces, e.g. medical equipment and is intrinsically resistant to antibiotics and disinfectants. Treatments of bacteria with an average initial concentration of (1.4±1.3)·10$^7$ CFU are performed in a 100 µl sample volume in a 96-well microtiter plate (300 µl) volume per well. A distance between the tip of the visible plasma and the liquid surface of 8 mm is maintained. The bacteria are inserted either in saline solution or distilled water and immediately after insertion treated with the plasma. Immediately after plasma treatment the bacteria are serially diluted (10 times) in PBS and plated on LB agar plates. After 24 hours incubation the amount of viable colonies on the plates are counted, yielding a quantitative measurement of residual bacterial density in CFU/ml with a detection limit of 10$^1$ CFU/ml. The data was analysed with the software SPSS Statistics and evaluated with the Mann-Whitney U test which showed a significant change between control and treated plates at indicated time points. Since the used solutions are not buffered the change of pH has been measured over time with a pH meter (VWR Symphony SB70P) in 2 ml distilled water and saline solution.
Figure 5.3: Log reduction after plasma treatment of *P. aeruginosa* in 100 µl of saline solution or distilled water as a function of treatment time. The experiments have been repeated at least 3 times. * Denotes significant difference compared to untreated control, *P* < 0.05, Mann-Whitney-U test.

5.3 Results and discussion

5.3.1 Bacterial inactivation

Figure 5.3 shows the logarithmic cell number reduction of the bacteria investigated as a function of plasma treatment time in distilled water and in saline solution. As expected, from literature (e.g. see [5–10]), a clear bactericidal effect of plasma treatment is observed. The magnitude of this effect depends on the treatment time but also on the type of bacteria and fluid composition. Note that the time necessary for similar log reductions for saline solution and distilled water is corresponding within a factor of 2.

Because the solutions are not buffered, the pH drops during the treatment. After a 20 min plasma treatment of 2 ml saline solution or distilled water, the pH reaches a value of 4.1. This would correspond to a proportional treatment time of 1 min for the 100 µl in which the bacteria are treated. A low pH alone is not sufficient to inactivate bacteria. Control experiments with bacteria in saline solution with a pH of 4.0 showed no significant bacterial inactivation at the same time scale as the plasma experiments. Bacteria in buffered solutions such as phosphate buffered saline (PBS) or sodium acetate solution are found to be less sensitive to
plasma treatment (data not shown), similar to the effects reported by Oehmigen
et al. [11].

The study of bacterial inactivation effects presented in figure 5.3 is complemented
by a treatment of bacteria in 3.35 ml saline solution which required around 13 min
for a log 3 reduction, which is a factor 2 to 3 faster than expected by assuming
a proportional treatment time. These measurements have not been repeated for
distilled water, since the inactivation rate by leaving the bacteria in distilled water
was of the order of $2 \cdot 10^5$ CFU/min. This is not significant for a 1 min treatment,
but becomes important for longer treatment times.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure5_4.png}
\caption{Axial gas temperature profile measured in the visible plasma using spatially re-
solved optical emission spectroscopy and using a type K thermocouple far downstream. The
surface of the treated liquid is positioned at 16 mm away from the end of the ring electrode. A
photograph of the plasma jet is shown to indicate the approximate position of the plasma.}
\end{figure}

5.3.2 Analysis of active plasma compounds

In the following the different plasma components are evaluated regarding their
possible contribution to the observed bactericidal effect.

Heat and gas flow

To investigate the influence of the gas flow alone on bacteria treatment, treat-
ments only with the gas flow on and the plasma switched off are done. It is found
to be harmless against bacteria in solution as prolonged exposure of the bacteria
in solution to the gas flow alone showed no detrimental effect on bacterial growth.
The gas temperature of the plasma varies with plasma dissipated power and gas type. Figure 5.4 shows that an argon plasma as described above has a gas temperature of approximately 80°C close to the visible tip of the plasma plume, which rapidly decreases as a function of distance. At the position of the liquid surface, the gas temperature is 45°C. In spite the elevated gas temperature, thermocouple measurements of the liquid do not show any significant temperature increase for the used treatment time.

The optimal growth temperature for \( P. \ aeruginosa \) is 37°C, but it will still replicate at a temperature as high as 45°C [12] and a temperature of approximately 60°C for a duration of 30 minutes is required to result in significant thermal induced bacteria kill [13]. The effect of flow and heat can thus be excluded for the bacterial inactivation.

**Electric fields**

The effect of the electric fields generated by the plasma treatment on cells in the liquid has been reviewed by Hülsheger et al. [14]. The survival rate of a bacterium as a result of electric field exposure is described by

\[
\hat{s} = \left( \frac{t}{t_c} \right)^{-\frac{(E-E_c)}{k}},
\]

where \( \hat{s} \) is the relative survival rate, \( E_c \) is the threshold value of field strength in kV/cm, \( t_c \) is the threshold value of treatment time in seconds and \( k \) is an additional factor. For \( P. \ aeruginosa \), the values of field strength, treatment time and the additional factor are approximately 6 kV/cm, 35 ms and 6.3, respectively [15]. However, as the plasma is not in contact with the liquid, no significant electric field is transferred from the plasma to the liquid phase which is the reason why the effect of electric field exposure is excluded in the following discussion.

**Metastable species**

As the plasma is produced in pure argon, significant production of metastables, atomic argon and dimer argon molecules is to be expected, which will produce reactive species in the downstream region of the plasma jet. As significant admixing of air is expected in the jet at approximately 5 mm from the tip of the visible plasma (van Gessel \textit{et al.} and Reuter \textit{et al.} [16, 17] found more than 10% air admixture at approximately 7 mm for similar condition and plasma jets, although the gas flows rates were a factor 2 to 3 higher) the argon metastables will induce additional chemistry in the gas phase and the metastable argon density will be significantly reduced when reaching the liquid as is also seen in jets operating in the "bullet" mode [18].
(V) UV radiation

The plasma produces UV radiation but the absorbance of the liquid has to be considered in the assessment of potential UV induced inactivation of bacteria.

The VUV emission and its possible bactericidal effect was measured by Brandenburg et al. [19] for a RF-Plasma jet with a slightly different setup. In this 20 W, 20 slm argon jet, the second continuum of the argon excimer (Ar$_2^*$) at 126 nm is found to be the dominant emission in the VUV range. By using windows that either transmit only UV (quartz) and both VUV and UV (magnesium fluoride), the difference in bactericidal efficacy was determined to be negligible. However later work by Lange and von Woedtke [20] where samples behind the filters were flushed with pure nitrogen showed that VUV radiation results in a higher bactericidal effect than UV treatment alone.

It has to be noted that these treatments were performed on polyethylene strips with dried bacteria and/or spores rather than in a liquid medium. Additionally, VUV with a wavelength smaller than 180 nm is completely absorbed by liquid layers which are thicker than 100 $\mu$m [21]. As such, in the treatments discussed in this work, any effect of VUV on bactericidal efficacy is due to the possible production of reactive species such as OH at the gas-liquid interface (see also further). As is known from photochemistry a significant flux of VUV on water can lead to significant dissociation of H$_2$O producing effectively OH at the gas-liquid interface. The quantum yield at 123.6 nm (corresponding to the emission of the Ar$_2^*$ excimer) is 1 [22].

Absolute radiance measurements have been performed on two different RF jets [19, 23] and show similar results in terms of VUV-radiance, i.e. 2.2 and 2.6 mW mm$^{-2}$sr$^{-1}$, respectively.

Estimations of the irradiance considering the area of the source, the area of the UV irradiated zone (1-3 times source area) and the distance of the jet to the water surface yield a photon flux (10.2 eV/photon) of $3 - 30 \cdot 10^{13}$ s$^{-1}$ and identical yield of OH production at the gas-liquid interface. It will be shown later that the upper value is approaching the required OH influx into the liquid to represent the observed liquid phase chemistry in the chemical kinetics model. VUV induced reactive chemistry in the liquid phase can thus not be excluded as a potential source of liquid phase radicals.

The measured UV emitted by the plasma jet shows that the lowest wavelength with a significant intensity is approximately 310 nm, as is seen in figure 5.5. As water does not strongly absorb these wavelengths, bactericidal effects could be induced.

Previous research concerning the bactericidal effect of UV sterilization by gas discharge lamps shows that a 1 log reduction in bacterial density is achieved after 40 seconds of treatment in case of P. aeruginosa using a low-pressure mercury
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Figure 5.5: Absolutely calibrated UV spectrum of a (1.4±0.1) W argon plasma.

A lamp with an irradiance of 1 W/m² on an initial population of 10⁶ CFU/ml [24]. The bacteria were treated in 2 or 15 ml amounts of suspension in which a 10 mm diameter opening was exposed to the UV radiation. Low pressure mercury lamps mainly emit at a wavelength of 254 nm (UVC) and are often used as bactericidal lamps as the absorption cross-section of DNA is known to be high at this wavelength [25].

Fernández et al. [26] investigated the effects of UVA on P. aeruginosa using a black light lamp, which has the highest emission at 365 nm with an irradiance of 44 W/m². The bacteria were treated in glass chambers with an internal diameter of 4.6 cm. The irradiation resulted in a 1 log reduction after approximately 20 minutes, and a 3 log reduction after 60 minutes.

Soloshenko et al. [27] made a comparison of water decontamination efficiency by using various UV sources such as a cold hollow cathode discharge as well as low- and medium-pressure mercury lamps. The used bacterium was E. coli. Their results show that bactericidal efficacy of UV is not only dependent on the absorption by DNA but depends also on the wavelength of the UV radiation. The above mentioned low-pressure mercury lamp produced a UV irradiance of 1 W/m² at approximately 254 nm. The irradiance of the plasma jet used in this work is almost three orders of magnitude lower, namely 2.9 mW between 200 and 400 nm. In addition the highest intensity is at 310 nm which is less bactericidal than UV radiation at 254 nm. Thus, the bactericidal effects of UV by damaging the DNA in the cells can be neglected under the presented treatment conditions.
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Ions

Figure 5.6 shows the mass spectra of positive and negative ions reaching a distance of approximately 5 mm from the tip of the plasma plume. The observed ions are all clusters, among others of \( \text{H}_3\text{O}^+ \), \( \text{CO}_4^- \), \( \text{H}_2\text{O}^- \) and \( \text{HO}_2^- \). This can be explained by the fact that any ions reaching the sampling orifice will have travelled a large distance through the argon/humid air atmosphere, which increases the ability of accumulating polar water molecules. The reason for cluster formation is that the initially created ions are transformed to the ions with the lowest ionization energy, for example:

\[
\text{Ar}^+ + \text{H}_2\text{O} \rightarrow \text{H}_2\text{O}^+ + \text{Ar},
\]

followed by

\[
\text{H}_2\text{O}^+ + \text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{OH},
\]

finally resulting in

\[
\text{H}_3\text{O}^+ + \text{H}_2\text{O} + \text{M} \rightarrow \text{H}_3\text{O}^+ (\text{H}_2\text{O}) + \text{M} \quad \text{and} \quad \text{H}_3\text{O}^+ (\text{H}_2\text{O})_{n-1} + \text{H}_2\text{O} + \text{M} \rightarrow \text{H}_3\text{O}^+ (\text{H}_2\text{O})_n + \text{M}
\]

These processes have been studied in detail in the context of ion measurements in the drift zone of DC coronas in air [28, 29].

In mass spectrometry measurements performed by Bruggeman et al. [30] on a water helium atmospheric pressure discharge \( \text{H}_3\text{O}^+ \) was also found, but there was no \( \text{H}_2\text{O}^+ (\text{H}_2\text{O})_k \) which corresponds to masses \( 18 \cdot k \). The reason for this is...
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the fast charge exchange (reaction 5.3) which will quickly convert H$_2$O$^+$ to H$_3$O$^+$ and its clusters. The presence of these masses in the measured mass spectrum for the RF plasma jet open to air could be attributed to NH$_4^+$ and its clusters [28].

For negative ions the most dominant clusters are H$_2$O$^-(H_2O)_n$ which are formed via

$$e^- + H_2O + M \rightarrow H_2O^- + M \quad (5.6)$$

and HO$_2^-(H_2O)_n$. While the neutral molecule of the latter is easily formed by

$$OH + O_3 \rightarrow HO_2 + O_2, \quad (5.7)$$

no electron attachment of this molecule is expected as it is observed to dissociate [31] or to ionise and form a positive ions [32]. Ions of the same mass have been observed before in oxygen-acetylene flames [33], where the possibility of it being CH$_2$O$^-$ is suggested. Carbon-based compounds in the form of CO$_2^-(H_2O)_n$ are also observed. Similar compounds have already been reported by Bruggeman et al. for a He-H$_2$O RF atmospheric pressure plasma at low discharge powers [30].

As a result, the most dominant expected reaction of positive cluster ions reaching the liquid surface is the recombination of the ion, potentially producing radicals such as OH or increasing the acidity of the solution. As such, any influence of incoming flux of positive ions is expected to be expressed in induced liquid chemistry rather than any direct effect on bacteria or cells present in the liquid.

Negative ions on the other hand show a more complicated behaviour when in contact with water, which is not completely understood. However, there are indications that negative ions have a propensity to adsorb at the surface of the liquid rather than penetrate into the bulk and absorb. This is explained by the significant dipole moment due to the the orientation of the topmost layer of water molecules [34, 35]. HO$_2^-$ for example is the conjugate base of H$_2$O$_2$. Thus, in an acid environment it can lead to additional H$_2$O$_2$ production. Because of the adsorption, negative ions are also not expected to react directly with bacteria or cells present in the liquid, again suggesting the important role of plasma induced liquid chemistry.

Van Gessel et al. [36] measured an electron density of approximately $10^{19}$ m$^{-3}$ (~10 ppm) inside the plasma plume generated by this plasma source. When taking into account dissociative electron-ion recombination which has a typical rate of $10^{-13}$ m$^{-3}$/s [37], the actual density of ions reaching the liquid surface is significantly (2-3 orders of magnitude) lower than the density in the visible plasma plume. Reactive species densities such as ozone, OH and NO as discussed in the
next section are significantly larger. Ion induced chemistry will therefore not be
dominant under the present conditions. Further analysis of these mass spectra is
outside the scope of this work.

5.3.3  Liquid chemistry induced by reactive species

As mentioned in chapter 1, Zhang et al. [38] measured ozone concentrations of
the order of $10^{21} \text{m}^{-3}$ using the same argon RF jet as used in this work with ad-
mixtures of $\text{O}_2$, while van Gessel et al. [39] measured nitric oxide concentrations
of the order of $10^{21} \text{m}^{-3}$ with the argon RF jet and admixtures of $\text{N}_2$. OH con-
centrations have been measured for a similar jet up to $10^{22} \text{m}^{-3}$ by Verreycken et
al. in the case of an Ar-$\text{H}_2\text{O}$ mixture feed gas et al. [40]. Assuming that similar
concentrations of reactive species are produced in the jet used in these studies, we
discuss in this section the influx of the reactive species on the chemistry in the bulk
of the liquid in order to gain insight into the mechanisms of for example nitrite,
nitrate and hydrogen peroxide generation as well as to find estimates of chemical
species densities possibly responsible for the observed bactericidal effects.

The fluid chemistry induced by the flux of ozone, nitric oxide and hydroxyl radicals
impacting the surface of the liquid is calculated as a function of time using zero
dimensional solution kinetics simulations. To this end, a system of 30 coupled
differential equations of the form

$$\frac{d[A]}{dt} = k_{BC} [B] [C] - k_{AD} [A] [D] - \ldots + \ldots$$  (5.8)

is solved, where $[A]$, $[B]$, $[C]$ and $[D]$ are arbitrary particle concentrations in units
M (mol/l) and $k_{BC}$ and $k_{AD}$ are second order rate constants for the components
mentioned in the subscript in M$^{-1}$s$^{-1}$. A positive term indicates the production
of the species $A$ whereas a negative term indicates the destruction of the species.
Aside from second order reactions, also first and third order reactions occur, in
which case the reaction rate constants are also of first and third order, having
units of s$^{-1}$ and M$^{-2}$s$^{-1}$ respectively.

The used reactions and their respective reaction rates are shown in table 5.1 and
are taken from the NDRL/NIST Solution Kinetics Database [41]. The relevant
reactions of the components present in and produced by the system are selected
to form a self-consistent system of reactions. A schematic visualisation of the
processes taken into account in the chemical model is shown in figure 5.7.

The starting concentrations of $\text{H}^+$, $\text{OH}^-$, $\text{O}_2$ and $\text{Cl}^-$ are taken to be approxi-
mately $10^{-7}$, $10^{-7}$, $3 \cdot 10^{-4}$ and $6 \cdot 10^{-6}$ M respectively. The starting values of OH$^-$ and $\text{H}^+$ are chosen because of an initial pH of 7.0. The concentration of oxygen
in the water is estimated by Henry’s law $^*$. Nitrogen (either N or N$_2$) is not a

\[ p = k_H c, \quad \text{where } p \text{ is the partial pressure, } k_H \text{ is the Henry constant and } c \text{ is the concentration.} \]

For oxygen in water $k_H \approx 769 \text{ atm-M}^{-1}$. 

---

$^*$ Henry’s law states that the solubility of a gas in a liquid is proportional to the partial pressure of the gas above the liquid. The constant of proportionality is known as the Henry’s law constant ($k_H$). For oxygen in water, $k_H$ is approximately $769$ atm-M$^{-1}$.
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Figure 5.7: Schematic of the processes included in the liquid chemistry model.

part of the calculations as it is not found to contribute to any relevant reactions in the NIST database. The initial nitrite and nitrate concentrations are defined to be approximately $10^{-5}$ and $5 \cdot 10^{-6}$ M respectively, as measured for untreated samples.

Finally a non-zero concentration of chloride is used because the distilled water used for the ion chromatography measurements still contained chloride. In spite of the fact that Na$^+$ will also be present in the water in similar concentrations, there are no applicable reactions of Na$^+$ in water in the NIST database and as such it is not included.

Motivated by the measured values in literature, as mentioned above, and since the chemical species density such as NO do not vary significantly in time even during the kHz modulation [39] a constant flux of O$_3$, NO and OH originating from the plasma is assumed, the values of which are determined by using an optimization algorithm to find values at which the end concentrations of nitrate and nitrite correspond to the values found in the ion chromatography measurements, namely $1 \cdot 10^{-4}$ M of nitrate and $8 \cdot 10^{-6}$ M of nitrite after 60 seconds of treatment. The externally induced production term in the bulk of the liquid of O$_3$, NO and OH required to achieve the experimentally measured nitrate and nitrite concentrations are found to be approximately $2.9 \cdot 10^{-6}$, $44.5 \cdot 10^{-6}$ and $10.4 \cdot 10^{-6}$ M/s respectively.

The choice for this relatively simple but comprehensive model versus a model including more species and reactions is motivated in order to keep the number of unknown input parameters as low as possible. Now those unknowns are limited to 3 fluxes (similar to the 3 plasma production terms in Grymonpré et al. [42]) still allowing to predict both the pH, H$_2$O$_2$ concentration and the dependencies of the NO$_3^-$ and NO$_2^-$ concentrations as a function of the treatment time.

Roeselová et al. [43] used molecular dynamics simulations to investigate the interaction of hydroxyl radicals at the air-water interface. A water slab of 30x30x100
### 5.3: Results and discussion

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Reaction</th>
<th>Reaction rate (M⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OH + NO → NO₂ + H⁺</td>
<td>1 · 10¹⁰</td>
</tr>
<tr>
<td>2</td>
<td>OH + NO₂ → OH⁻ + NO₂</td>
<td>1 · 10¹⁰</td>
</tr>
<tr>
<td>3</td>
<td>O₃ + NO₂ → O₂ + NO₃⁻</td>
<td>2.5 · 10⁷</td>
</tr>
<tr>
<td>4</td>
<td>OH + OH → H₂O₂</td>
<td>5 · 10⁹</td>
</tr>
<tr>
<td>5</td>
<td>OH + O₂ → O₂ + HO₂</td>
<td>1 · 10⁹</td>
</tr>
<tr>
<td>6</td>
<td>OH + HO₂ → O₂ + H₂O</td>
<td>7.5 · 10⁷</td>
</tr>
<tr>
<td>7</td>
<td>HO₂ + HO₂ → O₂ + H₂O₂</td>
<td>1 · 10⁸</td>
</tr>
<tr>
<td>8</td>
<td>HO₂ + NO → HO₂NO</td>
<td>3.2 · 10⁹</td>
</tr>
<tr>
<td>9</td>
<td>OH + OH⁻ → H₂O + O⁺</td>
<td>1.3 · 10¹⁰</td>
</tr>
<tr>
<td>10</td>
<td>H⁺ + NO₂ + O⁻ → OH⁻ + NO₂</td>
<td>3 · 10⁸</td>
</tr>
<tr>
<td>11</td>
<td>NO₂ + NO → N₂O₃</td>
<td>1.1 · 10⁹</td>
</tr>
<tr>
<td>12</td>
<td>NO + NO + O₂ → NO₂ + NO₂</td>
<td>2.3 · 10⁶*</td>
</tr>
<tr>
<td>13</td>
<td>OH + Cl⁻ → ClOH⁻</td>
<td>3.5 · 10⁹</td>
</tr>
<tr>
<td>14</td>
<td>ClOH⁻ + Cl⁻ → OH⁻ + Cl₂</td>
<td>1 · 10⁴</td>
</tr>
<tr>
<td>15</td>
<td>Cl₂⁻ + Cl₂ → Cl₃⁻ + Cl⁻</td>
<td>4 · 10⁹</td>
</tr>
<tr>
<td>16</td>
<td>OH⁻ + Cl₂ → ClO⁻ + Cl⁻</td>
<td>4.5 · 10⁹</td>
</tr>
<tr>
<td>17</td>
<td>NO₂ + Cl₂ → Cl⁻ + Cl⁻ + NO₂</td>
<td>2.5 · 10⁹</td>
</tr>
<tr>
<td>18</td>
<td>H₂O + NO₂ + NO → HNO₂ + HNO₂</td>
<td>2 · 10⁸</td>
</tr>
<tr>
<td>19</td>
<td>H₂O + NO₂ + NO₂ → NO₃⁻ + NO₃⁻ + H⁺ + H⁺</td>
<td>0.5 · 10⁸</td>
</tr>
<tr>
<td>20</td>
<td>H₂O + NO₂ + NO₂ → HNO₂ + NO₃⁻ + H⁺</td>
<td>1.5 · 10⁸</td>
</tr>
<tr>
<td>21</td>
<td>H₂O₂ + OH⁻ → H₂O + O₂ + H⁺</td>
<td>2.7 · 10⁸</td>
</tr>
<tr>
<td>22</td>
<td>O₂⁻ + NO → ONO₂⁻</td>
<td>5 · 10⁷</td>
</tr>
<tr>
<td>23</td>
<td>O₂⁻ + O₃ → O₂ + O₃⁻</td>
<td>1.6 · 10⁹</td>
</tr>
<tr>
<td>24</td>
<td>O₂⁻ + H⁺ → HO₃⁻</td>
<td>5.2 · 10¹⁰</td>
</tr>
<tr>
<td>25</td>
<td>O₃⁻ + H⁺ → O₂⁻ + OH</td>
<td>9 · 10¹⁰</td>
</tr>
<tr>
<td>26</td>
<td>O₃⁻ + OH → HO₂⁻ + O₂</td>
<td>8.5 · 10⁷</td>
</tr>
<tr>
<td>27</td>
<td>O⁻ + O₃ → O₅⁻</td>
<td>7 · 10⁹</td>
</tr>
<tr>
<td>28</td>
<td>O₅⁻ → O₂⁻ + O⁻</td>
<td>5 · 10¹⁰</td>
</tr>
<tr>
<td>29</td>
<td>O⁻ + O₃ → O₂⁻ + O₂</td>
<td>7 · 10⁸</td>
</tr>
<tr>
<td>30</td>
<td>H₂O + HO₂ + O₂⁻ → O₂ + H₂O₂ + O⁻</td>
<td>9.7 · 10⁹</td>
</tr>
</tbody>
</table>

Table 5.1: Reactions and reaction rates used in fluid chemistry calculations. * indicates third order reaction rate, (M⁻² s⁻¹) and ** indicates first order reaction rate, (s⁻¹). Reactions and reaction rates are taken from [41].
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Table 5.2: Comparison of the estimated values of ozone and nitric oxide with measured values at similar conditions measured with the mass spectrometer. *1.4 W, 20%, **1.6 W, 20%.

<table>
<thead>
<tr>
<th>Species</th>
<th>est. density [m$^{-3}$]</th>
<th>meas. density [m$^{-3}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_3$</td>
<td>$7 \times 10^{19}$*</td>
<td>$2.8 \times 10^{19}$**</td>
</tr>
<tr>
<td>NO</td>
<td>$10 \times 10^{19}$*</td>
<td>$4.8 \times 10^{19}$*</td>
</tr>
</tbody>
</table>

A containing 864 water molecules was used and a single OH radical was placed 15 Å above the surface of the water. A total of 250 trajectories were calculated using initial velocities obtained from the Maxwell-Boltzmann distribution at 300 K. Of these 250 simulations, 81 radicals were absorbed in the liquid bulk whereas 142 radicals were adsorbed at the air-water interface. The rest of the trajectories were either scattered, desorbed or deflected. In the fluid chemistry calculations shown in this work, all of the adsorbed OH radicals are assumed to react to form H$_2$O$_2$, leading to a constant production of hydrogen peroxide. As this flux of radicals is treated separately, the requirement on the OH flux in the gas phase more than doubles. While other molecular dynamics simulations [44, 45] show that the propensity to adsorb rather than absorb is also applicable to ozone (10 molecules adsorbed for every molecule absorbed), this contribution is considered to be negligible in the calculations as reaction (5) has a reaction rate which is almost two orders of magnitude lower than reaction (4) and the production term of O$_3$ is an order of magnitude smaller, as shown before.

Assuming a 1.5 slm (2.5 \times 10^{-5} \text{ m}^3/\text{s}) flow, 6.022 \times 10^{23} \text{ particles per mol (Avogadro constant)} and a liquid volume of 100 \mu l an estimation can be made of the minimum required reactive species density in the gas phase to achieve the bulk chemistry effects as measured. Assuming an even distribution of the various species in the gas flow, this yields for O$_3$, NO and OH the values $7 \times 10^{18}$ m$^{-3}$, $1 \times 10^{20}$ m$^{-3}$ and $3 \times 10^{19}$ m$^{-3}$ respectively. This corresponds to 0.3 ppm of ozone, 4 ppm of NO and 3.5 ppm of OH.

Comparing these results with the measurement results of NO and O$_3$ (see table 5.2) a good agreement is found. However, when considering the low solubility of O$_3$ in water, the O$_3$ density estimated in the model could be an underestimate. The OH density is relatively large compared to reported measurements. However, additional OH generation is possible at the liquid interface by photo-dissociation of water molecules due to the (V)UV originating from the plasma jet as estimated above from values obtained in literature. However due to the relatively large distance between the plasma tip and the liquid layer, the air in between will absorb a large amount of the VUV radiation. In addition H$_2$O$_2$ could already be formed in the gas phase and be transported to the liquid phase. Additional production of OH from H$_2$O$_2$ in the liquid phase is possible due to the UV flux (the dissociation threshold is 557 nm [46]), nonetheless the UV flux is smaller than the estimated VUV flux. Similarly the NO can already convert in the gas phase to HNO$_2$ and HNO$_3$ as detected by other groups by means of FTIR in the afterglow of surface barrier discharges in air [47, 48]. So in this approach the NO
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Figure 5.8: Calculated concentration build up of various chemical species in water as a function of time during a 60 sec treatment for a treatment volume of 100 µl.

The flux represents the total NO containing species \((\text{H}(\text{NO})_{(n)})\), including secondary products, while we assume in the calculation that the conversion only happens in the liquid phase.

Figure 5.8 shows the concentration in mol of various chemical species in the treated water as a function of treatment time on a double logarithmic scale. The treatment is limited to 60 seconds as for the antimicrobial treatment condition. The behaviour of the various concentrations after stopping the plasma treatment is shown in figure 5.9. A clear distinction is seen between species that act as an intermediate such as \(\text{O}^-\), \(\text{O}^\cdot\), \(\text{OH}\), \(\text{O}_3\), \(\text{HO}_2\) and \(\text{NO}\) and stable species such as \(\text{NO}^-\), \(\text{NO}^\cdot\) and \(\text{H}_2\text{O}_2\). Although \(\text{N}_2\text{O}_3\) is shown as a stable species in the simulation due to the reaction with water, it is an unstable species and vanishes via

\[
\text{H}_2\text{O} + \text{N}_2\text{O}_3 \rightarrow 2\text{HNO}_2. \tag{5.9}
\]

This reaction is not implemented in our calculations since no other significant reactions concerning \(\text{HNO}_2\) or \(\text{N}_2\text{O}_3\) are reported to be present.

To additionally check the validity of the calculated values, the concentrations of \(\text{H}_2\text{O}_2\), nitrite and nitrate for various treatment durations are calculated and compared to measured values. This comparison is shown in figure 5.10. The calculated concentration of nitrate as a function of time corresponds well to the almost linear measured behaviour. Nitrite calculations on the other hand, while showing somewhat different values, show the same trend. The maximum concentration of nitrite, calculated to be after approximately after 200 s/ml seconds of treatment, is in good agreement with the measurements. Hydrogen peroxide calculations show an agreement until 300 s/ml, after which the measured values are significantly higher. The concentration of \(\text{H}_2\text{O}_2\) is calculated to be 0.3 mM at 600 s/ml.
Figure 5.9: Calculated concentrations of various chemical species in water as a function of elapsed time after applying a 60 second plasma treatment for a treatment volume of 100 µl.

The measured H₂O₂ concentration at 600s/ml is approximately 0.6 mM, making the calculated value reasonably consistent with the experiments. The main difference is the quadratic behavior of the hydrogen peroxide concentration, probably due to an increase in water vapor concentration above the surface of the water over time leading to an increased production of OH and thereby H₂O₂. On the other hand, in the calculations, a constant production of OH is assumed. The first order components in both fits shown in figure 5.10(b) are comparable however, being 1.44 · 10⁻³ mMs⁻¹ in the measurements and 5.13 · 10⁻³ mMs⁻¹ in the calculations. The discrepancies between measurements and calculations of NO²⁻ and NO³⁻ might be because of slightly different or smaller reaction rates which are not included in the calculations. Although there are differences between the measurements and the calculations, these graphs provide an indication that the calculated fluid chemistry is a reasonable estimation for the species concentrations in the liquid bulk.

A simultaneous increase in concentration of both H⁺ and OH⁻ is observed while they recombine to form H₂O with a reaction rate of 3·10¹⁰ M⁻¹s⁻¹ [49] in accordance to the equilibrium expected from the pKₐ value of water. Considering the pH with and without this reaction yields 3.9 and 3.7, respectively. The former is in reasonable agreement with the measured pH value of 4.1. Since nitrite and nitrate are the main dominant negative ions in the liquid we can assume that the pH corresponds directly to the concentration of nitrate and nitrite [50]. This can be estimated with the following formula: \( pH = pK_a + \log(\frac{NO_3^-}{HNO_2}) \), with \( pK_a = 3.4 \) and assuming that all NO²⁻ is NO³⁻, which will be a maximum possible concentration. With a pH of 4.1 after a 20 min treatment time of 2 ml of liquid one gets a NO³⁻ concentration in the liquid phase of 0.08 mM. This leads to a HNO₂ concentration of 0.016 mM, which is in correspondence with the measured
5.3: Results and discussion

Figure 5.10: Comparison between calculations and measurements of \( \text{H}_2\text{O}_2 \), nitrite and nitrate concentrations as a function of treatment time for a volume of 3.8 ml and 3.35 ml, respectively. In the hydrogen peroxide figure, a third order fit of the function \([\text{H}_2\text{O}_2](t) = -4.68 \cdot 10^{-7}t^3 + 0.165 \cdot 10^{-3}t^2 + 1.44 \cdot 10^{-3}t \text{ mM}\) is shown in red for the measurement data and a linear fit of \([\text{H}_2\text{O}_2](t) = 5.13 \cdot 10^{-3}t \text{ mM}\) is shown in red for the calculated data. Both of the fitted functions have a fixed intercept at the origin.

(a) Nitrite (\(\text{HNO}_2\)) and nitrate \(\text{HNO}_3\)

(b) \(\text{H}_2\text{O}_2\)
value of HNO₂, i.e. 0.016 mM in 22 min (see figure 5.10).

The concentrations of the species discussed in the following sections differ by a maximum of a factor of 4 between including and excluding OH⁻ reduction. This is within the expected accuracy of the results obtained from this model, as in general acid dissociation reactions and self-ionization of water are both not accounted for in this model.

It is interesting to note that the above assumed surface production of hydrogen peroxide by recombination of OH radicals adsorbed at the water-air interface is essential to achieve the measured concentrations. When this surface production term is not included in the calculations, a hydrogen peroxide concentration of approximately 3.8 \times 10^{-8} \text{ mM} is calculated, i.e. an underestimation of almost seven orders of magnitude. Aside from surface production of H₂O₂, its formation in the gas phase (by reactions of OH with itself) should be also taken into consideration as an additional production term. However, a significant flux of OH still has to reach the bulk of the liquid to induce the chemistry observed in treatments. Because of this, surface production of OH might be still an important contribution.

In order to further validate the assumption of proportional treatment time hydrogen peroxide concentrations have been measured for different treatment times and volumes ranging from 100 µl to 4 ml and 1 to 40 min, respectively. Smaller volumes for the ion chromatography measurements could not be used resulting that nitrate and the nitrite measurements were not possible for the smaller volumes. The hydrogen peroxide concentrations showed a non linear behaviour resulting in a higher measured concentration, when assuming a linear relationship between treatment volume and treatment time, which is qualitatively similar to the results shown in figure 5.10(b). This indicates that the measured concentrations of hydrogen peroxide for a volume of 3.8 ml and a treatment time of 38 min are possibly an overestimate of the produced concentration in the 100 µl treatment volume and a 60 s treatment time but represent the right order of magnitude. The non-linearity of the hydrogen peroxide concentration as a function of time is explained by the increasing water vapour in the gap between the plasma and the liquid surface over time, which is more pronounced for larger treatment volumes. However, the same effect for NO related species is not expected as NO is formed in the core of the jet close to the nozzle [39], which is not affected (due to the gas flow) by an accumulation of the water vapour close to the water surface.

This means that the assumption of using a proportional treatment time can be at least used to obtain concentrations within an accuracy comparable to the model results.
5.3.4 Bactericidal components

In the remainder of this section the possible (unstable) bactericidal components and their concentrations and consecutive reactions are reviewed. At the end of the section the concentrations required to achieve bactericidal effects found in literature are summarized in table 5.3.

Peroxynitrous acid.

Peroxynitrite \((\text{ONOO}^-)\) manifests as being a stable species in the chemistry calculations. But it has been shown that \(\text{ONOO}^-\) is converted into peroxynitrous acid

\[
\text{ONOO}^- + \text{H}^+ \rightleftharpoons \text{ONO}OH, \quad \text{pK}_a \approx 6.8, \quad (5.10)
\]
a reaction which is dependent on the pH level. The concentration of peroxynitrite as a function of time is given by [51]

\[
\left[\text{ONOO}^-\right]_t = \left[\text{ONOO}^-\right]_0 e^{-kt}, \quad (5.11)
\]

where \(\left[\text{ONOO}^-\right]_t\) and \(\left[\text{ONOO}^-\right]_0\) denote the concentration of peroxynitrite as a function of time and the initial concentration respectively. The factor \(k\) is the pH and solvent dependent rate constant for the decomposition of peroxynitrite which is approximately 0.64 s\(^{-1}\) at a pH of 7.4 and 4 s\(^{-1}\) at a pH of 5.0 where all peroxynitrite is converted into peroxynitrous acid. This results in the half-life of \(\text{ONOO}^-\) being approximately 1 second in an environment with a pH of 7.4 and 0.2 seconds in a solution with a pH of 5. Because of this, the actual concentration of \(\text{ONOO}^-\) in the solution after treatment is expected to be negligible, as all of it is converted into peroxynitrous acid, which decomposes via [52]

\[
\text{ONO}OH \rightarrow f_1 (\text{OH} + \text{NO}_2) + (1 - f_1) (\text{NO}_3^- + \text{H}^+). \quad (5.12)
\]
The value of \(f_1\) is generally given as \(\geq 0.1\) [53], whereas Coddington *et al.* [54] proposes the reaction

\[
\text{ONO}OH \rightarrow f_2 (\text{OH} + \text{NO}_2) + (1 - f_2) (\text{HNO}_3) \quad (5.13)
\]

where \(f_2 = 0.29\) and is deduced from measurements of the formed oxidizing intermediate. The reason for low values of \(f\) is that the reaction products of the decomposition of \(\text{ONO}OH\) rapidly react with each other. Other possible reactions are [55]:

\[
\text{ONO}OH \rightarrow \text{NO}_2^+ + \text{OH}^- \quad (5.14)
\]
\[
\text{ONO}OH \rightarrow \text{NO}^+ + \text{OOH}^- \quad (5.15)
\]
\[
\text{ONO}OH \rightarrow ^1\text{O}_2 + \text{HNO} \quad (5.16)
\]
Producing among others $\text{OH}^-$, nitroxy (HNO) and singlet oxygen $^1\text{O}_2$. The production of singlet oxygen by reaction 5.16 however is calculated to be at least 23 orders of magnitude lower than the production of $\text{OH}$ and $\text{NO}_2$ in reaction (5.12) [56].

The bactericidal effect of peroxynitrite and its conjugate acid peroxynitrous acid are known from literature to be significant. Peroxynitrite is particularly toxic to cells due to its ability to diffuse through several cell diameters and cell walls before reacting. It is also able to initiate lipid peroxidation, causing cell wall damage [51]. ONOOH is a strong oxidant which reacts with biological molecules through various mechanisms. It is most efficient at oxidizing zinc fingers, protein thiols and iron/sulfur centers and it produces compounds that are normally expected of $\text{OH}$ reactions [57]. Zhu et al. [51] found that the bactericidal effect of peroxynitrite and peroxynitrous acid depends significantly on fluid pH level. A 4 log reduction of $\text{E. coli}$ was achieved by adding 1 mM of $\text{ONOO}^-$ in a solution with a pH of approximately 7.5. At a pH lower than 7.0 however, a reduction of barely one order of magnitude was achieved. This can be explained by the fact that even though the neutral peroxynitrous acid has the ability to travel through cell membranes and enter cells, where the pH is around 7.0, the resulting $\text{ONOO}^-$ reacts rapidly with $\text{CO}_2$ present inside the cells via [58]

$$\text{ONOO}^- + \text{CO}_2 \rightarrow \text{ONOOCO}_2^-, \quad (5.17)$$

which has a lifetime of approximately 1 μs and spontaneously decomposes:

$$\text{ONOOCO}_2^- \rightarrow \text{CO}_3^- + \text{NO}_2. \quad (5.18)$$

However, 65% of these radicals recombine immediately forming nitrate and carbon dioxide, resulting in only 35% being able to react with other molecules.

It has to be noted that the calculated maximum concentration of $\text{ONOO}^-$ (or $\text{ONOOH}$, depending on pH level) is approximately 1μM. As a result, the production of these species might result in a slight bactericidal effect at the beginning of the plasma treatment when the pH is high enough, and an insignificant bactericidal effect at a pH below 7.0. Also, the need for a high pH seems to directly contradict the observed increase of bactericidal effect at low pH values as observed in this work.

Conflicting views seem to exist on the actual decomposition reactions of peroxynitrous acid. The reactions shown above give a representation of reactions commonly found in literature. As reaction rates of these reactions are either not known, under debate or pH dependent they are not included in the calculations and remain for future investigations.
5.3: Results and discussion

Nitrous acid

The HNO₂ or nitrous acid (pKa=3.4), as calculated in the model, will decompose when in contact with hydrogen peroxide [59]:

\[ \text{H}_2\text{O}_2 + \text{HNO}_2 \rightarrow \text{HNO}_3 + \text{H}_2\text{O}, \] (5.19)

forming nitric acid. Alternatively, it can react as [60]

\[ \text{HNO}_2 + \text{H}^+ \rightleftharpoons \text{NO}^+ + \text{H}_2\text{O} \] (5.20)

\[ \text{NO}^+ + \text{H}_2\text{O}_2 \rightarrow \text{ONOOH} + \text{H}^+ \text{(slow)} \] (5.21)

Another possible reaction is [52]

\[ 2\text{HNO}_2 \rightleftharpoons \text{NO} + \text{NO}_2 + \text{H}_2\text{O}, \] (5.22)

where the forward rate is \(13.4 \text{ M}^{-1}\text{s}^{-1}\) [61] and the reverse rate is \(1.1 \cdot 10^9 \text{ M}^{-1}\text{s}^{-1}\). Note that the reverse rate is incorporated in the calculations, whereas the forward rate is not since the latter is many orders of magnitudes lower. After this reaction, nitrogen dioxide reacts via

\[ 2\text{NO}_2 + \text{H}_2\text{O} \rightarrow \text{H}^+ + \text{NO}_3^- + \text{HNO}_2, \] (5.23)

forming nitrate and nitrous acid. The result is a slight increase of nitrate and a decrease of nitrous acid concentration over a long period of time. Due to the pH dependent reaction between HNO₂ and NO₂⁻, a decrease in nitrous acid results in a similar reduction in nitrite at the same pH value. Because of the additional production of H⁺ in reaction (5.23), the effect is even more pronounced as more of the nitrite in the solution is present as HNO₂. This explains the time-scale and underlying mechanism of reduction in nitrite and increase in nitrate concentration after prolonged storage, an effect previously observed by Traylor et al. [62].

The generated NO₂ in reaction (5.12) can also be used to produce nitrous and nitric acid via [60]

\[ 2\text{NO}_2 + \text{H}_2\text{O} \rightarrow \text{HNO}_3 + \text{HNO}_2. \] (5.24)

Alternatively, nitric acid is produced by reaction [59]

\[ \text{OH} + \text{NO}_2 \rightarrow \text{HNO}_3. \] (5.25)

However, in acidic environment, nitrite is protonated to form nitrous acid:

\[ \text{NO}_2^- + \text{H}^+ \rightleftharpoons \text{HNO}_2, \] (5.26)

and it also forms due to the reaction of N₂O₃ with water (N₂O₃ is an unstable species) via equation 5.9 (Note that this reaction is not implemented in our calculations since no significant reactions concerning HNO₂ or N₂O₃ are believed to
be present) making the potential maximum concentration of nitrous acid approximately 1.3 mM when the calculated concentrations of HNO₂ and N₂O₃ and NO₂⁻ are added up (one N₂O₃ molecule results in two HNO₂ molecules).

Both nitric acid (HNO₃) and nitrous acid (HNO₂) have bactericidal properties. Nitrous acid is found to result in a 3 log reduction of *E. coli* at a concentration of 0.02 mM and a 7 log reduction at a concentration of 0.2 mM at a pH of 3.3 [63]. Even though the exact pathways of the decomposition of nitrous acid are unclear. Information about difference in sensitivity of *P. aeruginosa* to HNO₂ where not found in literature. Hence, the obtained values provide an indication that the bactericidal effect of plasma treatment is partly related to the toxicity of nitrous acid. The fact that a decrease in pH results in higher values of nitrous acid and therefore an increased bactericidal effect provides a possible explanation for the pH dependence found in plasma mediated inactivation of bacteria in this work, too.

**Hydrogen peroxide**

H₂O₂ can be a bactericidal agent provided the concentrations are high enough. At lower concentrations it may inhibit growth if the concentration is higher than the so-called minimum inhibitory concentration (MIC) [64], which depends on the bacterium. A synergistic effect of H₂O₂ with acidified nitrite was proposed by Naïtali *et al.* [65].

Another important aspect of H₂O₂ in contact with bacteria is that some bacteria are less susceptible to its effects when they were starved before treatment by being placed in a nutrition free solution (for example saline). Due to a decrease in cell metabolism, the bactericidal effect of hydrogen peroxide decreases. For *Peptostreptococcus anaerobicus* this effect is already visible after approximately 20 minutes of starvation [66].

**Ozone**

The bactericidal effects of ozone are known and industrially applied for many years. When dissolved, it slowly decomposes to oxygen with a half-life of approximately 20 to 30 minutes in distilled water [67]. Ozone can react with and oxidise organic compounds either directly or via a radical intermediates such as OH. In acidic solutions, O₃ is more stable than in alkali solutions [68]. As a result, the dominant deactivation mechanism in acidic solutions is the direct interaction of ozone molecules with cells. A further advantage of low liquid pH is the increase in solubility of ozone. The concentration of O₃ in water in an equilibrium situation is approximately 1.3 times higher at a pH of 4.0 than at a pH of 7.0 [68].

However, as the concentration of ozone in the solution without the plasma phase production term decreases rapidly after approximately 0.1 s the effect of ozone
can not explain any observed residual bactericidal efficacy after plasma treatment in our experiments. The reason for the short life time of ozone in the liquid is due to its fast reaction with nitrite and in addition due to the relative ineffective solubility of ozone in water (see above).

**Superoxide**

Previous research has shown indications of the influence of \( \text{O}_2^- \) on bactericidal efficacy [50, 69, 70]. It reacts as

\[
\text{HO}_2 \leftrightarrow \text{H}^+ + \text{O}_2^- , \quad (\text{pKa} = 4.88)
\]

(5.27)

depending on pH. The reaction thought to be responsible for its toxic effect is the so-called Haber-Weiss reaction [71]

\[
\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{OH} + \text{OH}^- + \text{O}_2 \,
\]

(5.28)

which forms OH and is thought to release oxygen in its singlet state in some cases [72]. However, normally the Haber-Weiss reaction does not occur without a catalyst such as Fe\(^{3+}\). It is suggested however that the process can still take place on the cell surface, which also allows for the resulting radicals to react with the cells before they decompose [73].

Quantification of the concentrations needed to achieve bactericidal effect is difficult. Superoxide is not assumed to be a contributing factor in the treatments shown in this work because of the low concentrations (\( \text{HO}_2 \) and \( \text{O}_2^- \) together constitute approximately \( 10^{-13} \) M) and the tendency to form \( \text{HO}_2 \) instead of superoxide at low pH levels. Also, most cells contain superoxide dismutases\(^\dag\), resulting in a conversion to oxygen and hydrogen peroxide. So while its neutral molecule \( \text{HO}_2 \) has the ability to penetrate cell membranes, it is rapidly converted by these superoxide dismutases [74].

**Summary of the liquid chemistry considerations**

As mentioned before, the maximum expected concentrations and the concentrations at which the individual components are bactericidal are shown in table 5.3. While these values are not for one specific bacterium, these serve as an order of magnitude estimation of the necessary individual concentrations of the various chemical species. The bactericidal concentrations of OH are quantified by photolysis of a \( \text{H}_2\text{O}_2 \) solution, but no other synergistic effects are considered at the reported values. The full comparison in table 2 yields that \( \text{HNO}_2 \) is likely to have an important effect on bacteria kill. Note that the found \( \text{H}_2\text{O}_2 \) concentration is large enough to inhibit bacterial growth. It is noted that the ONOO\(^-\) and ONOOH are likely to be abundant, although the latter has a short lifetime.

\(^\dag\)Enzymes that catalyse the dismutation of superoxide.
Table 5.3: Maximum estimated concentration (MEC) of various chemical species obtained from the measurements and the calculations and bactericidal concentration (bact. conc.), as reported in literature, including the corresponding references.

<table>
<thead>
<tr>
<th>Species</th>
<th>MEC (mM)</th>
<th>Bact. conc.</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ONOO</td>
<td>(9 \times 10^{-4}) (at high pH)</td>
<td>0.01 (pH 8.4) - 0.250 (pH 7.0)</td>
<td>[51, 75]</td>
</tr>
<tr>
<td>ONOOH</td>
<td>Unstable, can form NO and OH</td>
<td>10-10 (at low pH)</td>
<td></td>
</tr>
<tr>
<td>HNO</td>
<td>1.2 (at low pH)</td>
<td>0.2 (pH 3.3) - 30 (pH 5.0)</td>
<td>[52, 63]</td>
</tr>
<tr>
<td>H_2O_2</td>
<td>0.3 (calculated)</td>
<td>60 - 83</td>
<td>[76, 77]</td>
</tr>
<tr>
<td>O_3</td>
<td>0.0006</td>
<td>0.0046 to 0.1</td>
<td>[77, 81, 82], [67, 83, 84]</td>
</tr>
<tr>
<td>O_2</td>
<td>0.001</td>
<td>0.15 (pH 5.0) - 28, MIC</td>
<td>[64, 78–80]</td>
</tr>
<tr>
<td>HO_2</td>
<td>(10^{-10}) (at low pH)</td>
<td>Rapidly converted by superoxide dismutase</td>
<td></td>
</tr>
<tr>
<td>OH</td>
<td>(10^{-8})</td>
<td>(maximum in bulk during treatment)</td>
<td>[74]</td>
</tr>
<tr>
<td>NO</td>
<td>0.1</td>
<td>Approx. 4 ppm</td>
<td>[86, 87]</td>
</tr>
<tr>
<td>OONO</td>
<td>(10^{-9})</td>
<td>(maximum in gas phase during treatment)</td>
<td>[54, 84, 85]</td>
</tr>
</tbody>
</table>

Note: Concentrations are reported in brackets, and the corresponding references are included.
It should be noted that with increasing the treatment time to 120 s (results not shown) the model predicts an increase in NO\textsuperscript{−}, ONO\textsuperscript{−} and NO, while H\textsubscript{2}O\textsubscript{2} remains constant and O\textsubscript{3} even decreases. This illustrates the importance of reactive nitrogen species in the bacterial inactivation process.

We would like to stress that the literature values shown in the table are not specific for \textit{P. aeruginosa} but are provided as an order of magnitude impression of the bactericidal effect of the individual components.

### 5.4 Treshold of bacterial inactivation

Table 5.4: Example of a plasma treatment for different distances at a plasma power of 1.1 W and a treatment time of 75 s. The initial bacteria concentration in the liquid was 7 \times 10\textsuperscript{6} CFU/ml.

<table>
<thead>
<tr>
<th>Treatment distance [mm]</th>
<th>Log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>0.22</td>
</tr>
<tr>
<td>15</td>
<td>0.63</td>
</tr>
<tr>
<td>14</td>
<td>3.61</td>
</tr>
<tr>
<td>13</td>
<td>6.85</td>
</tr>
<tr>
<td>12</td>
<td>6.85</td>
</tr>
<tr>
<td>11</td>
<td>6.85</td>
</tr>
</tbody>
</table>

Figure 5.11: Transition distance as function of distance between visible plasmas tip and beginning of well plate and treatment time. The data points show average and standard deviation of 3 measurements.
To further investigate the chemical components responsible for the bactericidal effect, the log reduction of *P. aeruginosa* as a function of treatment distance, while keeping the plasma parameters identical, is obtained. By changing the distance while keeping the treatment time constant, a drastic change in log reduction when changing the treatment distance by 1 mm was found. The bacterial inactivation changed from a log reduction of 7, i.e. (nearly) complete inactivation, to a log reduction below 1 (see an example in table 5.4). We refer to the corresponding distance "threshold distance".

Figure 5.11 shows the average of the threshold distance (3 measurements for each data point) for different treatment times. It can be seen that the threshold is increasing with increasing treatment time. This sharp transition regime indicates that one or more components of the plasma, i.e. the components in the liquid produced by the plasma, drop at a certain distance below the bactericidal concentration.

Further measurements have been obtained for the previously analysed plasma components in order to compare the correlation of the change in the various plasma components with the change in threshold distance as shown in figure 5.11 and are summarised in table 5.5.

### Table 5.5: Summary of the distance dependency of the different components investigated in this work. The measurement of the hydrogen peroxide have been performed in 100 ml of liquid for 60s. The measurements of the pH have been performed in 2ml for 20min.

<table>
<thead>
<tr>
<th>Component</th>
<th>Range of change</th>
<th>conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$_2$O$_2$ (liquid)</td>
<td>(3.3 (\rightarrow) 1.0) mmol/l</td>
<td>1.0 W, (10.0 (\rightarrow) 16.0) mm</td>
</tr>
<tr>
<td>pH (liquid)</td>
<td>3.52 (\rightarrow) 3.64</td>
<td>1.0 W, (10.0 (\rightarrow) 16.0) mm</td>
</tr>
<tr>
<td>NO$_2^-$ (liquid)</td>
<td>(0.30 (\rightarrow) 0.23) mmol/l</td>
<td>calculated from pH</td>
</tr>
<tr>
<td>HNO$_2$ (liquid)</td>
<td>(0.23 (\rightarrow) 0.13) mmol/l</td>
<td>calculated from pH</td>
</tr>
</tbody>
</table>

As can be seen in table 5.5 the H$_2$O$_2$ does decrease with the distance, while the pH shows a very small increase. A slight change in pH indicates that the nitrite and nitrate concentrations do not depend significantly on the distance, which is to be expected, since the flux of NO is roughly independent of the distance to the nozzle (see chapter 4). The same holds for the HNO$_2$ in the liquid.

The sudden change in bactericidal effect with the distance between nozzle and liquid is a remarkable result, as it seems that the main change is the H$_2$O$_2$ concentration. To explain this in more detail, a comparison is made with the solutions prepared by Naitali *et al.* who found that acidified H$_2$O$_2$ (although in smaller concentrations as the present case 0.13 mmol/l) did not cause any significant bactericidal action, while acidified NO$_2^-$ (nitrate concentration 1.6 mM) leads to significant bactericidal action [65]. However the mixture of acidified NO$_2^-$ and H$_2$O$_2$ leads to even more bactericidal action suggesting a synergistic effect due to the formation of peroxynitrous acid by the reaction.
which is, as illustrated above, the formation of the peroxynitrous acid as a bactericidal molecule.

This is confirmed by further experiments of Boekema et al. [88]. In these experiments, various concentrations of H$_2$O$_2$ have been introduced into 100 µl of various solutions (i.e. LB with pH of 7, LB with pH of 4 and saline with a pH of 4). The bacteria are left in the liquid for the same time scales as with the plasma treatment, i.e. 30 to 120 s. As explained in section 5.2.4, directly after treatment the bacteria are diluted in PBS and plated on agar plates to quantify the amount of CFU/ml. It is shown that, at these treatment conditions, a significant log reduction is only observed at H$_2$O$_2$ concentrations higher or equal than 880 mM for all three investigated liquids. This is a factor 1000 higher than the hydrogen peroxide concentration detected after plasma treatment (see figure 5.10(b)). This is strongly suggests a synergistic effect between H$_2$O$_2$ and acidified NO$^-$ involved in the liquid chemistry, leading to bacteria inactivation.

5.5 Conclusion

The effects of heat, metastables, ion- and UV-fluxes, electric fields, and gas flow have been estimated to not have a prominent direct effect on bacterial inactivation by the remote RF atmospheric pressure plasma jet treatment under the experimental conditions being considered in this work. Any effect originating from these components is therefore due to their influence on the liquid phase chemistry.

The plasma treatment leads to a pH decrease in non-buffered solutions. The acidity of the treated solutions has a significant influence on the bactericidal effect. Buffered solutions are therefore incomparable to non-buffered solutions. The pH level determines the equilibria of various active chemical species such as peroxynitrite/peroxynitrous acid and nitrous acid but also the solubility of ozone which are known to be able to play a key role in bacterial inactivation.

The concentrations of the individual active components such as ONOO$^-$, HNO$_2$ and H$_2$O$_2$ derived from a combination of measurements and modelling are in the order of magnitude which would be necessary to observe bactericidal effects in solutions. A synergistic effect of some or all of the components is suspected to play a key role in the strong bactericidal effect of plasma treated solutions.

Reactive nitrogen species rather than reactive oxygen species (with the exception of H$_2$O$_2$) play an important role in bacterial inactivation under the present experimental conditions. The comparison of the liquid phase chemistry calculations and experimental measurements indicates the important effect of OH production and
its transmission into the gas-liquid interface with a corresponding production of H$_2$O$_2$. In addition the effect of VUV induced water dissociation at the gas-liquid interface is estimated to potentially contribute to the liquid induced chemistry.

Measurements of the different plasma components with varying distances between plasma tip and liquid surface have been obtained and indicate that the decrease of H$_2$O$_2$ correlates with the decrease of bactericidal action. This is ascribed to a synergistic effects between acidified NO$_3^-$ and H$_2$O$_2$ as previously suggested by Naitali *et al.*
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Abstract

Plasmas produce various reactive species, which are known to be very effective in killing bacteria. Plasma conditions, at which efficient bacterial inactivation is observed, are often not compatible with keeping human cells unharmed. The purpose of this study was to determine plasma settings for inactivation of *Pseudomonas aeruginosa*, without damaging skin cells in vitro under the same treatment conditions. An RF argon plasma jet excited with either continuous or time modulated (20 kHz, 20% duty cycle) voltages was used. To compare these two operation modes, only the input voltage was adjusted in order to obtain the same average power (1.7 W) for both modes. All other settings, i.e. gas flow, distance plasma tip to liquid surface, were kept constant. Bacteria or skin cells in physiological salt solution were exposed to direct non-contact plasma treatments. Short plasma treatments of up to 2 min resulted in a high reduction of bacterial numbers and did not affect dermal fibroblasts or keratinocytes. Bacterial inactivation has been previously ascribed to peroxynitrite, nitrite and H$_2$O$_2$ while eukaryotic cell viability is proposed to be reduced in the long term by the presence of H$_2$O$_2$ and is less affected by reactive nitrogen species. The remote RF plasma jet treatment was highly effective for bacterial inactivation while skin cell viability was preserved.

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6.1 Introduction

The purpose of the present study was to determine the settings of a radio-frequency atmospheric pressure plasma jet for inactivation of P. aeruginosa, without damaging eukaryotic cells. The specific plasma and plasma induced liquid chemistry for the source reported on in this chapter is reported in chapter 4 and chapter 5 and will be summarised below. This chapter is structured as follows. Section 6.2 introduces the experimental setup. In section 6.3 the results are presented and discussed. The chapter ends with a conclusion in section 6.4.

6.2 Materials and methods

6.2.1 Plasma source

A kHz modulated cold atmospheric RF plasma jet with a frequency of 13.56 MHz was used. The plasma source is described in the previous chapters. A schematic representation of the setup is shown in figure 6.1. The plasma was operated for 30 - 120 s with a gas flow of 1.5 slm of argon (purity 5.0). To reduce the gas temperature of the plasma, the RF voltage signal was modulated with a 20 kHz TTL pulse with a duty cycle of 20%.

A continuously driven plasma was tested for comparison. The average power, which was set by adjusting the input voltage, was (1.7 ± 0.1) W for both continuously and time modulated plasma. The surface of the suspension was always set at 8 mm from the plasma tip (see 6.1). Liquid phase H$_2$O$_2$ concentrations were measured with a colorimetric method [1]. NO and O$_3$ concentrations in the gas phase were obtained by molecular beam mass spectrometry with the system as described in chapter 4, with a detection limit of $2.5 \cdot 10^{18}$ m$^{-3}$ for NO and $2.5 \cdot 10^{19}$ m$^{-3}$ for O$_3$.

We showed in chapter 5 that the plasma jet in the remote treatment, as used in the present work, exerts its bactericidal effect through plasma induced fluid chemistry and not through (V)UV, electric fields, ions or heat flux. The effect of the flux of neutral species is significantly larger compared to the above mentioned contributions. Gas phase measurements have shown a large production of O$_3$ and NO from the jet [2, 3] and a significant production of hydrogen peroxide (H$_2$O$_2$), nitrate (NO$_3^-$) and nitrite (NO$_2^-$) anions has been measured in the case when distilled water was treated. The study reported in chapter 5 was complemented with a chemical model of the plasma induced liquid chemistry. It showed that the concentrations of the individual active components, such as ONOO$^-$, HNO$_2$ and H$_2$O$_2$, derived from a combination of measurements and modelling, were in the order of magnitude, which would be necessary to observe bactericidal effects in solutions.
6.2: Materials and methods

Figure 6.1: Schematic representation of the plasma source, which has a needle ring electrode geometry showing also the power supply, voltage and current probes, matching circuit, plasma source. The part of the plasma plume, visible to the naked eye, has a length of approximately 6 mm. The dimensions are not to scale and note that the inner diameter of the glass tube of the plasma jet is 1.65 mm and the inner diameter of the well is 6.6 mm.

6.2.2 Bacterial culture

*P. aeruginosa* (strain PAO1) was routinely cultured on Luria Broth (LB, Invitrogen, Paisley, UK) agar at 37°C. Bacteria from a logarithmic culture in 5 ml LB were diluted in LB, distilled water (DW), 0.85 % NaCl (NaCl), 0.85 % NaCl pH 7.8 (NaCl basic) or phosphate buffered saline (PBS, Invitrogen) to approximately 10⁷ colony forming units (CFU)/ml, based on OD₆₅₀. To quantify the CFU/ml before and after treatment, bacterial suspensions were serially diluted and plated on LB agar plates. The antibacterial effect of the treatment was calculated by the log reduction log(Nₜ/Nₖ), where Nₜ is the number of viable cells after treatment and Nₖ the number of viable cells in control samples.

6.2.3 Cell culture

Human dermal fibroblasts were isolated from the dermis as described in [4] and cultured in fibroblast culture medium (DMEM, supplemented with 10 % fetal calf serum, 1 mM L-glutamine (Invitrogen) and penicillin/streptomycin (Invitrogen). Fibroblasts were seeded in 96 well plates at a density of 4000-8000 cells/well and cultured until 80 % conflueny was reached.

Human keratinocytes were isolated from the epidermis as described [4]. Ker-
atineocytes were cultured in DMEM/Hams F12 (3:1) (Invitrogen), supplemented with 1% Ultroser G (Pall BioSepra, Cergy Saint-Christophe, France), 0.4 µg/ml hydrocortisone, 0.4 µg/ml isoproterenol, 5 µg/ml insulin, 1 ng/ml recombinant human KGF (all Sigma-Aldrich, St. Louis, MO), and penicillin/streptomycin (100 IU/ml penicillin, 100 µg/ml streptomycin; Invitrogen). Keratinocytes were seeded in 96 well plates coated with collagen IV at a density of 20,000 cells/well and cultured until 80% confluency was reached.

6.2.4 Plasma treatment methods

During treatment, the surface of the suspensions was always set at 8 mm from the plasma tip (see figure 6.1). The pH was measured over time with a pH meter (VWR Symphony SB70P) in 2 ml of solution in 12 well plates. As the size of the probe does not allow measurements of small volumes, a proportional treatment time was used to estimate the pH. 2 ml of solutions in 12 well plates were treated for up to 40 min. This would correspond to a proportional treatment time of 4 min for 100 µl in 96 well plates. Log reduction experiments comparing different volumes indicated that a 10 times longer treatment time was required for 2 ml samples.

Bacterial suspensions were treated in 96 well plates in 100 µl of 0.85% NaCl per well. Immediately after treatment, the suspensions were serially diluted in PBS, plated on LB agar plates and incubated overnight at 37°C to quantify the CFU/ml.

Fibroblasts or keratinocytes in 96 well plates were washed twice with 0.85% NaCl and 100 µl of 0.85% NaCl was added per well. Gas only treatment served as a control. Immediately after treatment, cells were washed twice with culture medium to remove any active compound and fresh medium was added. In this way, the cells were removed from the treated solution within 10 seconds after treatment, similar as for the bacteria. To estimate the amount of viable eukaryotic cells after treatment, MTT assays [5] were performed. Medium was replaced by the same medium containing 2 mg/ml MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Note that there is no direct plasma induced liquid chemistry effect on the MTT assay possible. Cells were incubated with MTT medium for 2 h at 37°C, 5% CO2. After discarding the medium, formazan in the cells was dissolved in 100 µl of DMSO and quantified in a spectrophotometer (OD560-OD550).

6.2.5 Statistics

Statistical analysis was performed with SPSS (Version 16.0 for MS Windows, SPSS Inc, Chicago, IL). The Mann-Whitney U (MWU) test was used to determine significant differences.
6.3 Results and discussion

Plasma treatment of bacteria in different media was performed to test the bactericidal activity of the source and its dependency on media (figure 6.2(a)). Significant bacterial inactivation was only observed in NaCl and DW, and not in PBS or LB. This appeared to be related to the buffering capacity of PBS and LB since plasma treatment lowered the pH of DW and NaCl to 4 but not of PBS or LB (figure 6.2(b)). Plasma treatment of NaCl with a pH of 7.8 (NaCl basic, obtained by adding 1 M NaOH to the NaCl solution) also resulted in a low pH, but only after longer treatment times (figure 6.2(b)). Moreover, bacterial inactivation was not observed in NaCl basic (figure 6.2(a)). This illustrates that the acidity of treated solutions has a significant influence on the bactericidal effect and that acidification is required for effective plasma treatment, similar to previously reported effects [6–9]. The pH level determines the equilibria of various active chemical species such as peroxynitrite/peroxynitrous acid and nitrous acid but also the solubility of ozone [10–12], which are known to play a key role in bacterial inactivation. The bactericidal effect of HNO$_2$ is also dependent on pH. Nitrite was shown to enhance bacterial killing at pH 2, to a small extent at pH 4 but not at all at pH 7 [13].

DW is not appropriate for testing plasma effectively because in DW i) the number of $P.\ aeruginosa$ without plasma treatment decreases with $10^5$ CFU/s and ii) the viability of eukaryotic cells decreases rapidly. Zone of inhibition test on agar plates were also not found to be appropriate because of poor quantification of bacterial inactivation. More importantly growth inhibition and inactivation cannot be separated when using agar plates. Therefore all further tests were performed in NaCl.

To improve bacterial inactivation, we compared kHz modulated (20% duty cycle) with continuous plasma at the same average pulsed plasma power (1.7 ± 0.1) W. Continuous plasma was more effective after shorter treatment times (figure 6.3). This suggests that the pulsing of plasma results in a different liquid chemistry and that (average) plasma power is of less importance. When the plasma is modulated, the electron density and e.g. the N$_2$(A) metastable density build up over several RF cycles so plasma properties are strongly modulated with time even during the plasma on time [2]. Therefore a different plasma induced chemistry is to be expected.

Significant differences in chemistry due to pulsing were observed for the pH and H$_2$O$_2$ in the liquid phase (table 6.1). The pH has an important effect on the concentration and bactericidal effect of HNO$_2$ [14], which is most likely one of the key species responsible for bacterial inactivation by the plasma. H$_2$O$_2$ alone was not sufficient for bacterial inactivation as it was well below the minimum inhibitory concentration (MIC) value for the used $P.\ aeruginosa$ strain, which we determined to be 490 mM. Time modulated plasma treatment of bacteria on agar plates has also been reported to cause a reduced inactivation of bacteria compared
Figure 6.2: (a) Log reduction after treatment of bacteria in indicated solutions with plasma (duty cycle 20%, 1.7 W). The average value of at least 3 experiments is shown. The error bars indicate standard deviation. Results from previous experiments with NaCl and DW shown in chapter 5 were replicated in the present study and combined to demonstrate differences in log reduction. Significant differences between DW and NaCl are indicated, MWU $P<0.05$ (*). (b) pH measurements after treatment of indicated solutions with plasma (duty cycle 20%, 1.7 W). 2 ml of solutions in 12 well plates were treated for up to 40 min. This would correspond to a proportional treatment time of 4 min for 100 µl in 96 well plates.
to continuous plasma [15]. The reduced O$_3$ concentration (below the detection limit for the used system) for continuous plasma (table 6.1), is most likely due to a higher plasma temperature and a lower O density (which leads to O$_3$ production in a 3 body reaction with O$_2$) in the core of the discharge.

Table 6.1: Effect of pulsing on pH, liquid phase H$_2$O$_2$ and gas phase NO and O$_3$ density at the treatment position. Plasma was operated at 1.7 W. *After treatment of 2 ml of NaCl in 12 well plates for 10 min. The average ± standard deviation of 3 measurements is shown. Initial pH was 5.83 ± 0.01. b After treatment for 1 min of 100 µl of NaCl in 96 well plates. The average ppm standard deviation of 3 measurements is shown. c Accuracy is ±1.5·10$^{19}$m$^{-3}$. *Significantly different from time modulated plasma (20 %), MWU P < 0.05.

<table>
<thead>
<tr>
<th>Duty cycle</th>
<th>pH$^a$</th>
<th>Liquid phase H$_2$O$_2$ $^b$ [mM]</th>
<th>Gas phase$^c$ NO [10$^{19}$ m$^{-3}$]</th>
<th>O$_3$ [10$^{19}$ m$^{-3}$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 %</td>
<td>4.23 ± 0.03</td>
<td>0.73 ± 0.04</td>
<td>6.05</td>
<td>&lt; 2.5</td>
</tr>
<tr>
<td>100 %</td>
<td>3.88 ± 0.03$^*$</td>
<td>1.24 ± 0.19$^*$</td>
<td>5.05</td>
<td></td>
</tr>
</tbody>
</table>

Since these plasma treatments were effective on bacteria, we tested the same settings on skin cells. Keratinocytes constitute the epidermis, while fibroblasts populate the dermis. Initial tests showed that prolonged incubation for 2 h of fibroblasts in the treated NaCl solution (with an equal volume of fresh culture medium added) significantly reduced cell activity (figure 6.4(a)). Time modulated plasma at 20 % duty cycle for 2 min reduced fibroblast activity after 2 h incubation...
tion to 18% of the control. To measure only the direct effect, cells were washed immediately after plasma treatment. This is similar to the tests for bacterial inactivation where bacteria were quickly diluted in PBS and plated. Using these conditions, the activity of fibroblasts was unaffected by time modulated or continuous plasma treatment up to 2 min (figure 6.4(b)). Exposure of keratinocytes in NaCl to either plasma also resulted in unchanged cell activity as measured by the MTT assay (figure 6.4(c)). Plasma treated cells were compared to cells treated with non-ionized gas. Values above 100% might indicate more cells present (variation in the number of cells at the start of the experiment) or a higher cellular activity. The cytosolic protein Lactate dehydrogenase (LDH) is commonly used to quantify membrane damage. LDH could however not be used under the present experimental conditions because we observed inactivation of LDH by the plasma treatment.

The relative resistance of eukaryotic cells to plasma treatment can be explained in various ways. To cope with oxidative stress in vivo, cells have a large repertoire of proteins such as superoxide dismutase or various peroxidases to scavenge for and neutralize RNS and ROS. The intracellular CO\(_2\) reacts faster with peroxynitrite to form ONOO\(\cdot\)O\(^-\) than the uncatalysed decomposition of peroxynitrite [16]. ONOO\(\cdot\)O\(^-\) can decompose via homolysis to NO\(_2\) and CO\(_3\)\(^-\). The effect of nitrite might be reduced by the presence of (intracellular) phosphate [17, 18]. Finally, the surface area and the surface to volume ratio might play a role as well. These explanations are consistent with the sustained cell viability for short treatment times.

The observed reduction in eukaryotic cell viability after prolonged exposure to plasma treated solutions is probably related to H\(_2\)O\(_2\). H\(_2\)O\(_2\) is relatively stable and reaches up to 1.25 mM after 1 min treatment. The concentration at which H\(_2\)O\(_2\) is cytotoxic for fibroblasts is dependent on the incubation time. We found H\(_2\)O\(_2\) to be cytotoxic at 479 M after 2 h and at 15 mM after 5 min incubation. A correlation between H\(_2\)O\(_2\) concentration and cell viability has also been reported recently for keratinocytes [19]. The increased presence of ozone for time modulated plasma might have contributed to the reduced viability of fibroblasts after prolonged exposure (figure 6.4(a)) as exposure of fibroblasts to ozone can result in significant mortality [20]. RNS do probably not affect viability of eukaryotic cells in the present experiment. Nitric oxide reached 6 ppm in the gas phase in our setup, while 2000 ppm was found to cause no change in viability of endothelial cells [21].
6.3: Results and discussion

Figure 6.4: Cell viability of fibroblasts (a and b) or keratinocytes (c) after treatment in NaCl with plasma at 20\%, 1.7 W or at 100\%, 1.7 W, or with argon gas. Viability of the cells was assessed by measuring their ability to produce formazan in an MTT assay. Results with gas only were set at 100\% for each treatment time. Cells were left in plasma treated NaCl solution plus an equal volume of culture medium for 2 h (a) or cells were washed immediately after treatment and kept in culture medium for 2 h (b and c) before the MTT assay was performed. Gas only did not affect cell viability. Extended incubation in plasma treated NaCl solution greatly reduced cell viability (a). Time modulated or continuous gas plasma did not affect viability of either cell type after immediate washing (b and c). Average values of 3 experiments are shown, bars indicate standard deviation.
6.4 Conclusion

Eukaryotic and prokaryotic cells in physiological saline solution were treated with a remote RF cold plasma jet. The chosen conditions determined that the plasma reactivity for biological interactions was conveyed through induced liquid chemistry. Plasma can be highly effective in reducing bacteria while at the same time plasma preserves skin cell viability for short treatment times. Bacterial inactivation has been related previously to peroxynitrite, nitrite and $\text{H}_2\text{O}_2$, while eukaryotic cell viability is proposed to be reduced in the long term by the presence of $\text{H}_2\text{O}_2$ and is less affected by RNS. Therefore a short plasma exposure allows for effective reduction of bacteria while eukaryotic cells remain viable in the same medium.
References


Chapter 7

Guided and branching streamers in DC nanosecond pulsed excited CAPPJs *

Abstract

Plasma bullets are ionisation fronts created in atmospheric pressure plasma jets. The propagation behaviour of those bullets is in literature explained by the formation of an interface between the inert gas and the ambient air created by the gas flow of the plasma jet, which guides these discharges in the formed gas channel. In this work we examine this ionisation phenomena in uniform gases at atmospheric pressure where this interface between two gases is not present. By changing electrical parameters and adding admixtures such as oxygen, nitrogen and air to the gas flow the conditions for which plasma bullets are present is investigated. Nanosecond time resolved images have been taken with an ICCD camera to observe the propagation behaviour of these discharges. It is argued that the inhomogeneous spatial concentration of metastable atoms and ions, due to the laminar gas flow and the operation frequency of the discharge in the range of a few kHz, is responsible for the guidance of the ionisation fronts. Furthermore, conditions have been observed at where the branching of the discharge is stable and reproducible over time in the case of a helium plasma by adding admixtures of oxygen. Possible mechanisms for this phenomenon are discussed.

*This chapter is a modified version of the publication: S. Hofmann, A. Sobota, and P. Bruggeman - Transitions between and control of guided and branching streamers in mono polar nanosecond pulsed excited atmospheric pressure plasma jets, IEEE Transactions on Plasma Science, vol. 40, no. 11, Nov. 2012
7.1 Introduction

To obtain more physical insight into the guided streamers in atmospheric pressure at kHz-frequencies and small excitation voltages (< 6 kV), for which the memory effect is essential to ignite a plasma, we are investigating the conditions at which the plasma bullet like behaviour can be obtained in pure argon or helium atmospheres and for which conditions the bullet propagation is inhibited and random branching is observed. Since the interface between two gases is not present in a uniform gas a guidance of the streamer discharge is not a priori expected yet observed in these and other experiments.

To understand this behaviour argon and helium plasma jets are excited by nanosecond DC pulsed discharges in an argon or helium atmosphere, respectively, and in air. We obtain nanosecond time resolved images to investigate the influence of parameters such as the gas flow, the voltage, the frequency and the amount of other gas admixtures on the propagation behaviour of the discharge. This enables us to determine the conditions for streamer branching.

The chapter is organised as follows. First the experimental setup and the measurement method is explained (section 7.2). In section 7.3 the results for argon are shown and the conditions for non-branching streamers in uniform gases are presented. We discuss and make estimates of the effect of long living metastable atoms and ions produced by the previous discharges on the memory effect and the branching and non-branching conditions. In section 7.4 conditions of stable branching in helium mixtures are reported and an analysis of possible mechanisms is presented. Finally the results are summarised in section 7.5.

7.2 Experimental setup and conditions

Figure 7.1 shows the schematics of the experimental setup. The plasma jet is mounted inside a flow-through reactor which is filled with either argon or helium at 1 bar. The pressure is regulated with a one-way valve. The inner and outer diameter of the glass tube are 1.8 mm and 3.0 mm, respectively. The diameter of the needle is 1.0 mm. In the case when air is used as a background gas the reactor is open during operation. If not indicated otherwise a flow of 1 slm of helium, argon with or without admixtures of nitrogen, oxygen or air are blown into the chamber through the glass tube.

To create the positive high voltage pulse a DEI PVX-4110 pulse generator is used for which the high voltage is supplied by a Spellman DC power supply. This allows to produce unipolar voltage pulses up to 10 kV with a full width at half maximum ranging from 170 ns up to several milliseconds with a maximum repetition frequency of 10 kHz. The frequency and duty cycle are controlled by a signal generator (Agilent 33220A LXI) which triggers both the pulse generator
7.2: Experimental setup and conditions

Figure 7.1: Schematics of the experimental setup.

and the delay generator controller necessary for the image acquisition. A 12.5 MΩ resistor is connected in parallel to the needle electrode for safety reasons to ensure that no residual charge remains at the needle after the operation. The mass flow of argon, helium (both with a purity of 5.0) and the mixtures are controlled with two mass flow controllers (BROOKS 5850 E and BROOKS Control unit 0254) with a maximum range of 10 slm and 300 sccm, respectively. The mass flow controller with the smaller range is used to add admixtures of nitrogen, oxygen, air, helium or argon to the gas flow. Voltage and current are obtained with a high voltage probe (Tektronix P6015A) and with a current monitor (Pearson 6656) which are read out by an oscilloscope (Agilent Technologies DSO 1024A, 200 MHz, 2 GSa/s).

Figure 7.2(a) shows a typical voltage and current signal as a function of time of the nanosecond pulsed DC argon discharge in an argon atmosphere at atmospheric pressure, at 5 kHz, 5.0 kV and a pulse width of 500 ns with a gas flow of 1 slm. Comparing the current with the voltage it can be seen that the current is mainly displacement current due to the capacitive nature of the setup. From these V-I traces an estimate can be made of the energy per pulse. Indeed from the displacement current an estimate of the capacitance ($\approx 5 \, \text{pF}$) is obtained. The applied voltage (i.e. 5 kV) yields the energy stored in this capacitor, which is about 50 $\mu$J. The integral of the first current peak gives an estimate of the charge initially going in the discharge and is of the order of 25 nC.
Chapter 7: Guided and branching streamers

Figure 7.2: Voltage and current waveforms of the argon and the helium discharge.

(a) Argon, 5 kHz, 5.0 kV, 500 ns pulse width.

(b) Helium, 5 kHz, 4.6 kV, 500 pulse width.
The voltage current waveforms for all the other cases are similar to the one in figure 7.2(a), except for the case of pure helium, where a large voltage amplitude (> 3.5 kV) resulted in a plasma discharge in the tube as well as around the tube to the wall of the grounded chamber. In this case a significant plasma current is also observed (figure 7.2(b)) leading to an overestimate of the charge density for pure helium.

For the time resolved images an ICCD camera (Andor) is connected to a delay generator (Stanford research systems DG535). To focus and magnify the plasma, a lens with a focus of 30 cm is placed between the ICCD camera and the chamber. A photographic lens is attached to the camera. This configuration allows that a length of 16.6 mm is imaged on the CCD. The trigger of the camera is synchronised with the delay generator controller.

An exposure time of 10 ns is used, if not indicated otherwise. We changed the gain and the shutter time (between 0.1 s to 2 s) of the camera for the images in order to get a reasonable signal since single shot images often did not provide enough light for a reasonably good image for the smaller applied voltages. This means that images are accumulations of typically between 500 and a few thousand pulses. If branching occurred in the case of argon the branching looks diffuse on the images, but single shot measurements with an exposure time of 30 ns revealed the branching characteristic for the larger voltages. Since the main parameter we want to observe is the morphology rather than the intensity of the plasma every image is adjusted to the highest contrast.

Two examples of single shot and accumulated measurements are compared in figure 7.3. The experimental conditions are of the argon discharge as described in figure 7.2(a) (figure 7.3(a)) and at a higher voltage of 6.0 kV (figure 7.3(b)), while the other parameters are kept fixed. The gray scale in this figure as in all other images are black and white inverted (black = highest intensity). The black lines on the images indicate the position of the inner edge of the glass tube (inner diameter 1.8 mm) and the needle, respectively. Comparing these two images it is clear that in figure 7.3(a) only one emission peak is visible at the axis of the needle, while for higher voltages branching is visible as seen in figure 7.3(b). In figure 7.3(d) this branching is visible as a diffuse off axis emission due to the accumulation of the random branching. An off axis diffuse emission is thus an imprint of a filamentation.
Figure 7.3: Comparison of single shot measurements with accumulated measurements in the case of pure argon at 5.0 kV and 6.0 kV. The black lines indicate the position of the glass tube and the needle. In the case of figure a and b the contrast is enhanced for clarity.
7.3 Argon and admixtures

7.3.1 Air environment versus argon atmosphere: guided streamers in (in-)homogeneous gases.

In figure 7.4 we present time resolved images of an argon plasma in an argon atmosphere and in air both at atmospheric pressure. The voltage parameters are the same as indicated in figure 7.2(a). The shutter time and gain of the camera is adjusted for every plasma condition. The delay time is indicated in the images. The initiation of the plasma started at a delay time of around 130 ns. The reason of this delay is due to the cable length, the ICCD and the HV-pulse generator electronics.

(a) Argon in air. Gain: 220 V. Accumulations: 5000

(b) Pure argon. Gain: 220 V. Accumulations: 2500

Figure 7.4: Time resolved measurements of an argon plasma with a gas flow of 1 SLM, a voltage of 5.0 kV, a frequency of 5 kHz and a pulse width of 500 ns. The exposure time is on all images 10 ns. The gain and the on-chip integration time, $t_{\text{int}}$, (corresponding to $t_{\text{int}}/f$ accumulations) is varied for the images to compensate for the changes in intensity of the different discharges.

Stable propagating plasma bullets are created in both conditions. In the case for argon in air the discharge starts at the needle and moves to the edge of the glass tube. Afterwards the plasma leaves the tube at the edges of the glass tube. This is visible as two emission peaks which merge together a few millimetres further away and form a single propagation front (around 330 ns). This results in a ring-like structure of the radial emission of the discharge as observed in previous works by [1–3] and simulated by [4, 5] for a helium jet in air. The ring-like structure and guidance of the bullets are explained by the mixing of the carrier gas with the air, especially the nitrogen molecules around the channel, at the edge of the channel of the gas flow, since at this position the Townsend coefficient is still as high as in pure helium but significantly more ionisation occurs and $N_2(C)$ is produced (see also [6]).

In argon the same explanation is valid but additionally in this case emission from the decay of $N_2(C)$ is especially stimulated by a near resonant energy transfer
between argon metastables of the \( ^3P_{0,2} \) states and \( N_2(X) \) producing \( N_2(C) \) [7].

For the pure argon plasma the discharge grows as well towards the glass tube. However, the plasma does not leave the tube at the edge of the glass in this case but from the needle axis. Since the position of the emission is for the same delay time roughly the same for the plasma in air and in argon, the propagation velocity seems to be the same and equals approximately 30 km \( \cdot s^{-1} \). From figure 7.4 it is clear that under these conditions branching is not visible in both cases. Naidis found that for concentrations of air > 0.1% most of the electrons are lost by attachment. This will leave a larger source of charge on the edges of the tube surrounding next to the core compared to the centre. As the field during the development of the streamer is large enough to detach electrons from negative ions, in the case for ambient air, a preferential ionisation on the edge rather than in the bulk as in the case for the pure argon can exist.

The reason for the guided streamer in argon in the ambient air is clearly due to the interface between argon and air created by the gas flow. It is less evident that also in pure argon a guided streamer is observed since in this case the jet is mounted in an argon filled chamber. If the gas composition is homogeneous, the species produced by the previous discharges need to be primarily responsible for the guidance of the streamers.

Treating the bullet as a charged sphere with an estimated radius of 0.5 mm we estimated the reduced electric field \( E/N \) at the needle at 5 kV to be around 400 Td, which is in correspondence with other model results on streamers and plasma bullets [5, 8–10]. In the local field approximation this corresponds to a lower limit of the electron temperature of 9 to 10 eV, obtained from Bolsig+ [11] for the streamer head. In figure 7.5 the argon and argon metastable direct ionisation rate coefficient is plotted as a function of the electron temperature. For electron temperatures higher than 8 eV direct ionisation of ground state argon atoms is the dominant ionisation process of these two species if the ratio between metastables and ground state density \( n_{Arm}/n_{Ar} \) does not exceed \( 10^{-2} \) which is not expected for these conditions.

Note that in the cases of a two ring electrode system around a dielectric tube, these assumptions are not valid, since the electric field will be significantly reduced due to the lack of a sharp needle tip as the streamer emanates from a DBD discharge [12]. This leads to a lower electron energy making stepwise ionization more important than the direct ionization of the ground state atoms.

As the frequency of the discharges is 1 to 10 kHz we estimate the losses of the metastables and ions due to diffusion and chemical reactions. Assuming axial symmetry we estimated the time-scale of diffusion, \( t_{diff} \), during the pulse-off time in the following way,
7.3: Argon and admixtures

Figure 7.5: Direct ionisation reaction rate of argon and argon metastable atoms as a function of electron temperature at atmospheric pressure for a metastable and argon ion density of $10^{21}$ m$^{-3}$ and $10^{20}$ m$^{-3}$, respectively. The rate coefficients are calculated in Bolsig+ [11].

\[
\begin{align*}
  t_{\text{meta}} &= \frac{r^2}{2.4^2 \cdot D_{\text{meta}}} \approx 6.25 \text{ ms}, \\
  t_{\text{ion}} &= \frac{r^2}{2.4^2 \cdot D_{\text{amb}}} \approx 5 \text{ ms}.
\end{align*}
\]

$D_{\text{meta}}$ is the diffusion coefficient which is $6.7 \cdot 10^{-6}$ m$^2$/s in the metastable case [13] and $D_{\text{amb}}$ the ambipolar diffusion coefficient for molecular argon ions which is $8.4 \cdot 10^{-6}$ m$^2$/s assuming $T_e = T_g = 300$ K (the ambipolar diffusion coefficient is calculated with mobility values from [14, 15]), and $r$, the estimated emission radius of the streamer, approximately is taken as 0.5 mm. This shows that the spatial distribution of the species does not change significantly during the pulse off time which shows that the radial metastable or ion concentration distribution can cause the guidance of the bullets, at least if they are not lost due to chemical reactions. To account for the collision chemistry a simple axially symmetrical 1d kinetic model, including diffusion, is created to get an order of magnitude estimation of the density reduction of metastables and ions during the off-time of the pulse:

\[
\frac{\partial}{\partial t} n_i(r, t) = \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial}{\partial r} D_i \cdot n_i(r, t) \right) + S_{i, \text{prod}} - S_{i, \text{loss}},
\]

\[(7.3)\]
with \( n_i, D_i, S_{i,\text{prod}} \text{ and } S_{i,\text{loss}} \) being the densities, diffusion constants, production rates and loss rates of the species used in the model. The species, main reactions and reaction rates were obtained from [16] and [17] by assuming that the electron temperature equals the gas temperature (0.025 eV) and omitting reactions \( R_1, R_2 \) (rate constant small due to small electron temperature), \( R_7, R_8, R_{10}, R_{11} \) (production of \( \text{Ar}_2(1\Sigma_u) \)) is one order of magnitude lower than the production of \( \text{Ar}_2(3\Sigma_u) \)). Note that we also included the dissociation by atom impact by using equation (26) from Jonkers et al. [17]. The diffusion constants are the same as mentioned above. The diffusion constant of the argon dimer is assumed to be similar to the diffusion of the metastable argon atom and ambipolar diffusion is assumed for the charged species. In addition to that electron loss due to the three body process of attachment with oxygen molecules originating from the impurities of the bottle (< 6 ppm) with the help of argon atoms with a rate of \( 3 \cdot 10^{-14} \text{ m}^3/\text{s} \) is included. An estimate of \( n_e \) is obtained in the case of argon from the charge of the capacitance of the setup (25 nC) and the estimated volume of the discharge which yields \( n_e \approx 10^{20} \text{ m}^{-3} \). The detailed measurement of \( n_e \) is presented in chapter 8.

For the starting conditions a Gaussian profile of the included species is assumed with a FWHM of 0.5 mm, which is the estimated radius of the streamer. The initial electron density is the above estimated \( n_e \). The other initial densities are chosen as \( n_{\text{Ar}_m} = 10 n_{\text{Ar}_+} = 10 n_e \), neglecting argon ions and argon dimers at the start of the simulation. The 10 times larger metastable density compared to the electron density is motivated by model results from Sobota et al. [8].

In figure 7.6 the results of the simulation are shown. It can be seen that after 0.1 \( \mu \text{s} \) the charged species have the highest concentration compared to the other species taken into account in this model (figure 7.6(a)). The spatial profile, seen in figure 7.6(b), is flatter than in the beginning due to the higher losses at the centre, yet due to the slow diffusion the calculated FWHM (i.e. 1.9 mm) is still of the order of the inner diameter of the glass tube (i.e. 1.8 mm). This leads to the conclusion that the channel consisting of charged species will have a strong influence on the guidance of the streamers. To investigate this further we changed the frequency, the voltage, the gas flow and added admixtures of nitrogen, oxygen and air inside the gas flow. With these parameter changes we do observe transitions from guided streamers to branching streamers.

Figure 7.7 shows images of the argon plasma in an argon atmosphere for different gas flows, amount of admixtures, a higher voltage amplitude and a higher frequency. The reference parameters are 5 kHz, 5.0 kV, 1 slm of argon and a pulse width of 500 ns. In figure 7.7 all of the parameters but one are kept constant for each image. The changed parameter is indicated in the label of the images.

It is clear that branching occurred in all but 2 occasion, namely by adding 10% of \( \text{N}_2 \) and 10% of \( \text{He} \). In the following sections the different parameters and its effect on the morphology of the discharge are discussed.
7.3: Argon and admixtures

(a) Time resolved particle evolution in the centre of the bullet.

(b) Spatial resolved particle profiles at t = 0.2 ms.

Figure 7.6: Simulated particle density profiles as a function of time at the centre of the axis as a function of radial distance from the needle at the start of the voltage pulse as produced by the previous discharge.
Chapter 7: Guided and branching streamers

(a) Change of flow, frequency and voltage.

(b) Addition of 0.6% of nitrogen, oxygen, air and helium.

(c) Addition of 10% of nitrogen, oxygen, air and helium.

Figure 7.7: The argon plasma for different conditions. The reference settings are the same as in figure 7.4(b). The changed settings are indicated in the label of the pictures, all other settings are as in 7.4(b). The image is taken at a delay time of 330 ns if not indicated otherwise. The black lines indicate the position of the needle and the glass tube.

7.3.2 Effect of flow

With no flow branching is observed starting at the tip, which results in a typical corona discharge where the strongest branching is often observed at the high voltage needle at atmospheric pressure [18]. It shows that a flow is required to guide the ionisation front. This can be explained as follows. Since no flow is applied to the gas and diffusion of metastables and ions is quite slow compared to the period between two pulses (see above), the metastable and ion density remains relatively large in the vicinity of the needle. This allows to create very rapidly a strong and dense plasma in the high field region close to the needle which has a tendency to cause constriction and induce filaments and branching.

The images without flow, however, still show some kind of reproducible bullet propagation. This confirms that a memory effect is indeed present, meaning that the ionisation front formed during one pulse still gives a favourable direction for the streamers in the next few pulses.

A flow of 1 slm blows the metastables and ions approximately 2 mm further away at the time the next pulse starts. In this case the density of metastables and ions will be higher in front of the glass tube rather than in the vicinity of the needle.
due to the gas flow. This will reduce the effective ionisation rate (and thus charge density) of the initial plasma in the high electric field region of the needle which inhibits branching close to the needle. This phenomenon has also been observed by Xiong et al. however no explanation was given [19].

While a flow of 1 slm creates the guided streamers a flow of 4 slm shows branching again with no bullet propagation. Kim et al. [20] and Mohamed et al. [21] presented similar findings with their argon jet in air and reported the influence of a turbulent flow, while Li et al. [22] saw also a drop in the length of a helium plasma jet, due to an increased gas flow, resulting in a transition from a laminar to a turbulent flow. The Reynolds number for argon in the current investigated jet is approximately 1120 and 4480 for argon with a gas flow of 1 or 4 SLM, respectively. A flow of argon is in general laminar if the Reynolds number is below 2300 and turbulent if the number is above 4000 [23]. This shows that at 4 SLM turbulence occurs which inhibits the creation of a stable channel of ions and metastables and inhibits the creation of the guided streamer while creating random branching in the bulk. Comparing the two images with no flow and with a flow of 4 SLM it can be seen that in the first case the branching seems to start at the tip while for the high gas flow, the branching is more pronounced outside the tube.

7.3.3 Effect of frequency and voltage

By increasing the applied voltage the tendency for branching is increased. Due to the higher electric field and the subsequent increase of the ionisation rate streamers have a tendency to branch. A higher branching probability for higher voltages has been also observed in streamer research (see section 7.1). Although applied voltages are much larger in this case (10 - 50 kV) and the repetition rates of the pulses are significantly smaller (order of 1 Hz).

We obtained images at different voltages and frequencies to determine the voltage at which branching starts. It can be seen that increasing the frequency seems to lower the upper limit of the branching-voltage. We observed a hysteresis effect during these measurements, meaning that the highest voltage for which the branching started was not the same as the lowest voltage for which the branching was no longer observed. Figure 7.8 shows the voltages where branching starts to be observable. If the frequency was lower the plasma intensity was weaker and branching and non branching was harder to determine. Frequencies higher than 10 kHz could not be used, due to limitations of the high voltage pulse generator.

Even though the scattering of these findings is relatively large a negative trend for increasing frequencies is observed. The large scattering of data points is due to the fact, that the behaviour of the discharges depended strongly on the geometry and any small change in the needle during the experiments leads to changes in the experimental results.

The negative trend on the branching voltage is consistent with the fact that at
higher frequencies more argon ions (and metastable atoms) remain present from
the previous discharges in the surrounding of the needle. This clearly illustrates
that a memory effect is present as larger pre-ionisation and metastable density
lead to a stronger and faster ionisation which enhances filamentation/branching.
This shows that to obtain the so called plasma bullets one has to have enough
pre-ionisation to cause the memory effect but a too high pre-ionisation increases
the ionisation rate too much which leads to branching.

7.3.4 The effect of impurities

In figure 7.7(b) 0.6% of oxygen, nitrogen, helium and air are introduced in the
flow of the plasma. Comparing these four images it can be seen, that all of them
exhibit branching. Adding admixtures of a few percent to the discharge will
change, among other things, mainly two parameters: the Townsend ionisation
coefficient and the metastable density. A higher Townsend coefficient for the
same reduced field indicates a higher possibility of branching, due to the increase
of created charged species, while lower values would indicate the opposite.

In the argon-helium mixture bullet propagation is not inhibited but branching
occurs as well. Usually a higher electric field or a higher Townsend coefficient can
lead to a higher ionisation rate leading to streamer branching. The fact that in
these conditions the plasma behaves similar as increasing the voltage does strongly
indicate the influence of the Townsend coefficient. Quenching of metastables due to helium will not be as significant as in oxygen and nitrogen.

On the other hand, the quenching rate coefficient of metastable argon atoms by collision of nitrogen or oxygen molecules is in the range of $10^{-16}$ to $10^{-15}\text{m}^{-3}\cdot\text{s}^{-1}$, which is at least two orders of magnitudes faster than the reaction rate coefficient of metastable production. This means that by adding nitrogen, oxygen and air, the amount of argon metastables will be significantly reduced at the start of the next voltage pulse. In spite the fact that the ion density is larger than the metastable density, when the next voltage pulse is applied it is shown by Sobota et al. [8] that even when the metastable direct ionisation is not part of the dominant process it can still strongly influence the ionisation rate.

In the case of oxygen and air, not only metastables will be reduced but also the amount of electrons as well due to the electron attachment to oxygen which is reported by Naidis to be one of the main losses of electrons [5]. For small admixtures the formed negative oxygen ions can increase the effective ionisation rate significantly due to electron detachment at the next voltage pulse.

However it has to be noted that by adding admixtures the amount of impurities originating from the gas bottles and gas tubes increases as well. This is certainly an important factor for small flow rates, and can also contribute to the reduction of metastable and/or ion densities, especially in the case of mixing helium.

We increased the amount of admixtures to 10% and obtained images of the discharge which is shown in figure 7.7(c). Adding up to 10% of nitrogen in the argon atmosphere results in a guided streamer, but the velocity of this streamer is higher, i.e. by adding 10% of nitrogen causes the streamer to reach approximately the same position that the streamer in pure argon reaches 60 ns later.

By increasing the amount of nitrogen other reaction mechanisms become dominant. One of the processes that have not been included is the creation of nitrogen metastable molecules such as $\text{N}_2(A)$. Since $\text{N}_2(A)$ is not quenched by $\text{N}_2$ ground state molecules (unlike in the case of argon metastables which are lost by excimer formation) and the diffusion of the nitrogen metastables is in the order of the diffusion of the argon species, densities of metastables can be higher than in the case for pure argon [24].

The opposite is visible by adding 10% of air and oxygen. For these amounts of air and oxygen admixtures, electron attachment will reduce the direct ionisation coefficient as shown by Naidis [5], which could explain the reduced emission in these cases. Comparing the addition of 10% of helium with the addition of 0.6% of helium it is rather strange that there is such a big difference. It seems logical to presume that indeed impurities originating from the bottle at small admixtures caused the branching. Since the excitation energy of helium is much higher than the excitation energy of argon meaning that small admixtures of helium should not greatly alter the discharge.
7.4 Helium

In order to obtain a more thorough understanding of the branching we used helium as carrier gas in a uniform helium atmosphere to compare it with our findings with argon. Figure 7.9 shows time resolved images of a helium plasma in a helium atmosphere (figure 7.9(a)) and of a helium plasma in open air (figure 7.9(b)). The parameters are indicated in the captions. The camera settings are the same as in the previous images for argon.

![Time resolved images of helium plasma](image)

**Figure 7.9:** Time resolved measurements of a helium plasma with a gas flow of 1 SLM, a voltage of 4.06 kV and a frequency of 5 kHz.

In the pure helium case it can be seen that at a delay time of 330 ns the discharge is homogeneous and diffuse. Single shot measurements confirmed the diffuse character of the discharge, in contrast to the filamentary or bullet like discharge observed in argon. Using the same approach as in argon we estimate the electron density of the helium discharge to be smaller than $10^{17} \text{m}^{-3}$ (see section 7.2). This is 10 times lower than the electron densities in the case for the helium guided streamer in air as reported by Naidis [5]. This is in the range of capacitive coupled homogeneous helium RF-glow discharges, which basically can exist at atmospheric pressure because the Raether-Meek criterion is not satisfied at breakdown (which is not the case in argon) [25]. In the beginning of the discharge in pure helium and especially at a delay time of 170 ns the light emission is the lowest at the centre of the needle which is an indication of the inhomogeneity of the plasma in the flow zone.

Comparing the position of the highest emission for certain time delays, it can be seen, that the helium plasma in air is propagating faster than the diffuse front in the pure helium case. The higher velocity in the air environment is due to the smaller diameter of the ionisation front, compared to the pure helium discharge. A link between the velocity and the streamer diameter has been shown to exist by Luque *et al.* [10]. It is shown that a larger streamer diameter results in a lower electric field at its head, therefore resulting in a lower streamer velocity. The slow
propagation and low electron density motivates that in the pure helium case the ionisation front does not have the typical properties of a streamer.

A change of frequency, flow rate, voltage and low impurity concentrations did not significantly change the appearance of the discharge, which is the reason why these results are not presented here. Figure 7.10 shows images for different amount of admixtures as indicated in the figure.

By mixing argon the diffuse discharge changes to a more contracted plasma due to the typical larger electron density in the argon plasma (see chapter 2). This creates again the radial confined bullets. The same can be observed by adding nitrogen which has a similar effective ionisation rate than argon, which leads, for concentrations of 10% to the plasma bullets again. This is also observed by Wu et al. by adding 1.5% of nitrogen [26].

Adding 10% of O\(_2\) in the helium flow the discharge changes from a diffuse plasma to two reproducible propagating ionisation fronts leaving the nozzle. The evolution of the branching over time is shown in figure 7.11 in the case of the helium plasma with 10% of oxygen at 10 kHz and 6.0 kV. These parameters have been used since the stable splitting of two branches was best observed in this range. In these images the branching of the bullets is clearly visible. Compared to the random branching for argon with and without the admixtures, the branching or splitting observed in this case is stable in time and space, meaning that at the same delay time the ionisation fronts are at the same position.

Figure 7.12 shows effective Townsend coefficients of the different mixtures as a function of reduced electric field. The graph shows that the Townsend coefficient is lower if oxygen or nitrogen is added, making the channel of the gas flow in this case the least favourable. The splitting could be attributed to the fact that the pure helium gas in the surrounding is easier to ionise than the helium mixed with the admixtures in the centre of the discharge since due to the oxygen in the centre of the flow the Townsend coefficient is higher at the off-axis of the flow. This indicates that in this case inhomogeneities induce the branching, either due to a higher concentration of O\(_2\) and the electron attachment with oxygen in the core of the gas channel or because of inhomogeneities created by the flow.

Note that the branching occurs outside the tube for which the diameter of the streamer is significantly larger than the initial diameter. This will reduce the field
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to approximately 200 Td or less which is close to the region where the effective Townsend coefficient of pure helium is higher compared to the coefficient of helium with the admixtures of nitrogen, oxygen and air. The reason why this does not happen in argon is due to the smaller diameter of the ionisation in argon. Since the ionisation front is smaller than the tube diameter in the case for argon, the streamer is less influenced by the species outside the channel of the gas flow. The ionisation front of helium however is much larger, which means, that in the case when oxygen is added to helium the streamer can still propagate outside of the helium-oxygen channel through the area with less oxygen concentration.

Figure 7.11: Time resolved images of the helium discharge with 10% of oxygen at 10 kHz and 6.0 kV.

Brockhaus et al. [27] observed branching of an argon discharge similar to our configuration, yet this branching seemed to appear randomly. No explanation of this effect was given. Wang et al. [28] observed a similar bifurcation behaviour for their helium-oxygen DC plasma microjet, but did not present time resolved measurements. They explain the observation of this splitting due to the transition between a laminar to turbulent flow.

In our case turbulence cannot be the reason for the creation of the stable branching as the images of admixtures of 10% of nitrogen with helium show. In that case branching is also observed when N₂ is introduced in the flow inside the pure helium chamber which can be seen in figure 7.13, but became a non branching guided streamer after a few minutes, as shown in figure 7.10. This clearly indicates that the spatial gas composition and chemistry is the reason for the stable branching.

In the case for 10% of oxygen only a change of intensity over time was visible, meaning that due to the higher concentration of oxygen in the chamber, quenching of excited species and electrons increased. However the stable branching
behaviour does not change over time as it does when nitrogen is added. This was somewhat contradictory to our expectations since if the inhomogeneity is the only reason for this bifurcation, one would expect that the branching would disappear when the environment of the chamber would be the same as the gas flow, hence helium with 10% of oxygen. On the other hand a memory effect will be present due to the previous discharge, especially due to the negative ions, which will fix the path of the discharge once it is formed. When the plasma was ignited after the oxygen was added, the stable splitting however was still visible. To have a complete understanding of this effect an extensive model will be necessary. Nonetheless the stable and reproducible branching is a potential very suitable tool to study the branching phenomenon by more extensive diagnostics than mere imaging.

Figure 7.12: Effective ionisation coefficient per gas density for pure helium and the different admixtures. Obtained with Bolsig+ [11].

Figure 7.13: Time resolved images of the helium discharge with 10% of nitrogen at 5 kHz and 4.6 kV. A few seconds after adding the impurities.
7.5 Conclusion

In this chapter the morphology of a nanosecond pulsed atmospheric pressure argon and helium plasma jet with excitation voltages below 6 kV is presented. These types of plasma jets produce guided streamers (or plasma bullets) when operated in an air environment due to the guidance effect of the noble gas channel in the surrounding air. In this chapter the effect of the transition from a guided streamer to a branching streamer is investigated and the effects on this transitions of the gas flow, repetition frequency, gas composition and the related chemistry is presented.

We found that in uniform gases branching is inhibited and a guided streamer is formed only for specific parameters. The formation of a guided streamer is determined by the memory effect due to the species produced in the previous discharge. Calculations have shown that the channel of ionic species and metastables is still present at the time when the next voltage pulse starts, because the relevant loss processes and diffusion are slow. This allows for the guidance of the subsequent streamer. Experimental results such as frequency dependence and a hysteresis effect of the threshold voltage for branching support this conclusion. Turbulent flow causes mixing of the ion and metastable channel and induces branching while no flow causes a strong ionisation at the needle tip and immediate branching as in ordinary high voltage corona discharges. This allows us to conclude that a gas flow is a necessity to obtain a guided streamer.

Adding 0.6% of oxygen, nitrogen and air to the argon discharge flow results in branching and inhibition of a guided streamer. For large concentrations of nitrogen a guided streamer is again observed while the branching remains in the case of oxygen. This indicates that even though ionisation due to metastables is not the dominant ionisation process it seems that quenching of metastables induces branching. When oxygen is present, negative ion formation can explain the observed behaviour.

In helium, bullets and branching are only observed when the helium is mixed with a molecular gas or argon. In the case of pure helium a slowly growing homogeneous discharge is observed. When 10% of oxygen is added to helium, reproducible branching both in time and space is observed, which shows a possibility to control branching and allow in depth experimental studies of the phenomenon.
References


Chapter 8

Electron densities and energies of a guided argon streamer in argon and air environments

Abstract

In this study we report the temporally and spatially resolved electron densities and mean energies of a guided argon streamer in ambient argon and air obtained by Thomson laser scattering. The plasma is driven by a positive monopolar 3.5 kV pulse, with a pulse width of 500 ns and a frequency of 5 kHz which is synchronized with the high repetition rate laser system. This configuration enables us to use the spatial and temporal stability of the guided streamer to accumulate a multitude of laser/plasma shots by a triple grating spectrometer equipped with an ICCD camera and to determine the electron parameters. We found a strong initial $n_e$-overshoot with a maximum of $7 \cdot 10^{19}$ m$^{-3}$ and a mean electron energy of 4.5 eV. This maximum is followed by a fast decay towards the streamer channel. Moreover, a 2D distribution of the electron density is obtained which exhibits a peculiar mushroom-like shape of the streamer head with a diameter significantly larger than that of the emission profile. A correlation of the width of the streamer head with the expected pre-ionization channel is found.

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8.1 Introduction

Streamer corona discharges have been studied for decades motivated due to a large range of applications including gas exhaust treatment re-mediation, water cleaning, dust collection and surface modification of materials [1, 2]. Particularly pulsed streamers have been shown to have the ability to be very efficient for re-mediation of small amounts of impurities in gases due to the efficient production of reactive species and radicals [3]. As streamers are filaments which are highly transient in time, have a minimum diameter of the order of 100 $\mu$m [4] and in addition are produced randomly in space, their physical properties have mainly been investigated by imaging, emission spectroscopy and electrical characterization [5–7]. During the propagation phase of the streamer, the electrons in the head of the streamer have high energies which drive both the ionization and dominate the production of excited species and thus the emission of the streamer. As a consequence time resolved imaging yields mainly information on the propagation of the streamers and modelling has and is playing a key role in understanding other properties of these discharges [8–11]. The reactive species (such as e.g. OH) produced by the streamer have been investigated by laser diagnostics [12, 13]. In many cases volume averaged species densities are obtained due to the random nature of the streamers or experimentally demanding single shot laser diagnostics need to be performed.

To avoid the spatially random nature of the streamers several approaches have been investigated to stabilize streamers. Brandenburg et al. [14] have used spatially stabilized filaments in a DBD discharge configuration to allow detailed studies of the emission spectra by cross correlation spectroscopy. This approach allows to study breakdown processes in detail and e.g. to obtain temporally and spatially resolved maps of the reduced electrical field during the discharge development. Similar work has also been performed for a surface discharge by Simek and co-workers [15].

In the last decade, atmospheric pressure plasma jets have received a lot of attention in the plasma community. The jets are typically generated in a DBD-like configuration in a tube in He or Ar and can propagate along the helium or argon jet in ambient air [16, 17]. For dc pulsed or sinusoidal voltage excitation at kHz repetition rates, it has been found that the dynamics of the jets, as revealed from their time resolved emission, appear as bullet-like plasma volumes travelling with velocities in the range of $10^4 - 10^6$ ms$^{-1}$ [16, 18]. It has been shown that these plasma jets are guided streamers which are very reproducible in space and time [19, 20]. In the previous chapter we showed that it is possible to produce an argon guided streamer in an argon environment with pulsed dc excitation. This is ascribed to the spatially dependent pre-ionization when the guided streamers are generated with a repetition rate in the kHz range. The same configuration as in chapter 7 is used in the current work.

Temporally and spatially resolved electron density and energy measurements in
streamers have not been performed before. Line broadening of the hydrogen lines are in principle possible as typical electron densities are reported to be in the range of \(10^{20} - 10^{21} \text{m}^{-3}\). However, the complication arises that the space-charge field contributes to the line broadening [21]. As in liquids the electron densities are typically larger, streamer-like discharge have been studied in more detail with line broadening [22, 23].

In this work, Thomson scattering (TS) is used, because it is a direct measurement of the electron properties which avoids lengthy interpretations prone to uncertainties such as with line broadening [24]. In addition it can be performed with a resolution which is determined by the detector and the laser beam waist down to 50 \(\mu\text{m}\) and has excellent time resolution up to a few nanoseconds. This technique has recently also been applied to MW plasma jets [25, 26], dc excited microplasmas [27] and RF plasma jets [28] at atmospheric pressure.

We will show the temporally and spatially resolved distributions of \(n_e\) and the mean electron energy \(\langle E_e \rangle\) of an Ar plasma jet generating a guided streamer in ambient argon and air. A strong initial \(n_e\)-overshoot with a maximum value of \(7 \times 10^{19} \text{m}^{-3}\) and a mean energy peak at 4.5 eV is observed, followed by a fast decay. Strong differences which are observed between the electron density profile and the emission profile will be discussed. It is noted that the work found in literature on Ar streamers is rather limited [4, 29, 30], which limits the possibility to compare our findings with previous published results.

### 8.2 Setup

The employed setup is the same plasma jet as used in chapter 7. The jet is operated in a closed vessel at 950 mbar. The glass tube with the gas flow and the needle electrode is mounted on an x-y stepper-motor stage. A positive voltage of 3.5 kV and an Ar gas flow of 1 slm is applied. The pulsing frequency is 5 kHz and the pulse width of the rectangular pulse is 500 ns with a rise time of 50 ns. The inner diameter of the glass tube of the jet is 1.7 mm and the diameter of the needle is 1 mm. For more details the reader is referred to chapter 7.

Thomson scattering is performed with a second harmonic Nd:YAG 532 nm laser. The laser has a repetition frequency of 5 kHz, a (FWHM) pulse width of 8 ns and a pulse energy of 4 mJ. The laser beam is aligned across the plasma plume and passes the chamber through Brewster windows. The scattered photons are detected by a triple grating spectrograph equipped with an ICCD (Andor iStar) camera under a scattering angle of 90°. Consequently a 2D image is obtained, where one axis corresponds to the spatial dimension along the laser beam (in this case the radial direction of the jet) and the other axis resolves the spectral information. A 1D image of \(n_e\) and \(\langle E_e \rangle\) in the radial direction of the streamer is obtained. For details of the TS setup, the reader is referred to van de Sande [31] and for the experimental handling to van Gessel et al. [25].
Figure 8.1: Photograph of the plasma jet together with an overlay of the plasma emission, taken by an ICCD camera, of an argon guided streamer with slightly different plasma conditions than used in this work (taken from chapter 7). The emission is taken with an exposure time of 10 ns at about 200 ns after the voltage pulse starts. FWHM of the emitting channel is about 0.5 mm.

The temporal resolution of the measurements is given by the ICCD gate, which corresponds to a minimum of 2 ns, and the spatial resolution is determined by the triple grating spectrometer, which corresponds in this particular case to 50 µm. The detection limits are mostly determined by the laser stray light and are in the order of: $n_e > 10^{18} \text{ m}^{-3}$, $\langle E_e \rangle \approx 0.15 - 10 \text{ eV}$. It is ensured that the laser energy does not have any influence on $n_e$ nor $\langle E_e \rangle$ obtained in this work. The results are verified not to depend on the laser pulse energy in the range of 0.8 to 4 mJ.

**Measurement procedure**

First, the laser is spatially and temporally aligned with respect to the plasma. With the help of stepper motors the laser beam was always ensured to be in the centre of the plasma jet. A delay generator shifts the laser with respect to the voltage pulse in time and the plasma can be scanned time-resolved by TS. After some initial delay time the plasma is found to be reproducible in space and time, so multiple laser shots can be accumulated for a single measurement. From each TS raw image the laser stray light at plasma ”off” is subtracted. At the temporal peak of the streamer, Bremsstrahlung continuum emission from the plasma is observed which is also subtracted. The raw TS data can be seen in figure 8.2 for different time steps. Note that for each time step a 1D radial profile is obtained.
Between 150 and 155 ns the streamer head is reaching the position of the laser,

\[ \delta \lambda = 9 \text{ nm} \]

which results in an increase of intensity of the Thomson signal. In ambient argon at 165 ns two zones with high electron density off-axis can be seen in the images, between 170 ns and 180 ns three distinct zones are visible while at later times only the actual streamer channel in the centre causes a TS signal.

To allow a precise determination of the electron density and mean electron energy as a function of time (and space), the measured TS intensity is binned in a range of 40 pixels (≈ 0.5 mm) to provide a better signal-to-noise ratio. This width corresponds roughly to the streamer channel diameter. In order to obtain the absolute electron density the TS signal is calibrated with the rotational Raman laser scattering in nitrogen at room temperature and 500 mbar. For more details of the absolute calibration the reader is referred to the work of van Gessel et al. [25].

8.3 Results

First the time resolved values of \( n_e \) and \( \langle E_e \rangle \) from the streamer channel in the case of ambient Ar and air are presented. Subsequently the time-resolved 1D mapping capability of the TS measurements is used to present the 2D profile of the electron density in the streamer channel and head in the case of the Ar guided streamer in ambient argon.
This section deals with the central streamer channel, of which the FWHM is around 0.5 mm. TS was performed on an argon jet in ambient argon at 950 mbar. The time resolved electron density was recorded for three different axial positions as shown in figure 8.3. For the axial position at $z = 3$ mm, (where $z = 0$ corresponds to the end of the glass tube of the plasma source), three different exposure times were used. The electron density at the initial peak of the streamer is found to be around $n_e = (7 \pm 1) \cdot 10^{19} \text{m}^{-3}$ and decreases slightly with larger distance to the quartz tube to a density of $n_e = (3 \pm 0.5) \cdot 10^{19} \text{m}^{-3}$ at $z = 17$ mm. The arrival time delay of the streamer at different positions is related to the propagation velocity of the streamer. Close to the tube a velocity of 48 km/s is measured while further downstream a velocity of 40 km/s is found which is in agreement with the emission measurements obtained in the previous chapter. After the initial peak, the electron density decays very fast with 1/e-times of 15 ns, after this initial drop the time scale of the decay is in the range of 100 – 200 ns. For the position close to the tube when the voltage on the needle is switched off after 500 ns, a re-ignition of the plasma is observed.

The different exposure times of the TS measurements reveal no fundamental differences in the results, however, it is clear that any exposure time smooths the results in time. Any jitter in the ignition of the plasma will also smooth the results, consequently the reported values of $n_e$ at the head of the streamer might be lower than the real maximum values.

**Figure 8.3:** Electron density as function of time at three different axial positions for the Ar streamer in Ar.
8.3: Results

Figure 8.4 shows the corresponding mean electron energy in the streamer channel for three different positions and again for the first position for three different exposure times. The maximum of $\langle E_e \rangle$ is found to be around $4.5 \pm 1$ eV for all positions. Subsequently the electrons cool rapidly with $1/e$-times of 15 ns towards an energy of 0.3 eV. The latter value is constant in the streamer channel and lasts as long as TS can be observed.

A re-ignition at the end of the voltage pulse, so a rising $\langle E_e \rangle$ is observed, corresponding to the plasma emission from this plasma source for the closest axial position to the tube as found in chapter 7, also seen in [32, 33]. That might be due to the discharging of the dielectric tube after the voltage is removed from the needle electrode [34]. The other two positions at 10 and 17 mm are apparently too far away to show significantly re-ionization by the voltage drop.

![Figure 8.4: Mean electron energy as function of time at three different axial positions for the Ar streamer in Ar.](image.png)

### 8.3.2 Argon streamers in ambient air

The argon plasma jet was also operated in ambient air. The last three images in figure 8.2 represent the Thomson signal obtained for the argon-air plasma. To overcome the strong Raman scattering signal from the surrounding air, the outer parts of the 1D detection strip were blocked, leaving an image of around 0.8 mm. However, still a Raman signal from the air entrainment is visible at the top and bottom. If the Raman scattered photons would not be blocked, their intensity on the ICCD would strongly overshadow the TS signal. That leads to the conclusion
that it is not possible to measure \(n_e\) and \(\langle E_e \rangle\) in the outer parts of a streamer channel in air by the presented method for the experimental conditions under investigation.

**Figure 8.5:** Electron density for argon in argon and argon in air as function of time.

**Figure 8.6:** Mean electron energy for argon in an argon and air environment as function of time.
8.3: Results

In figure 8.6 the electron density of the argon in argon streamer is compared with $n_e$ of the argon in air streamer at 3 mm away from the glass tube. The first impression is that the results in ambient air do not differ much from ambient argon as also found in chapter 7. The velocity of the streamers is similar and for 3 mm downstream from the tube, the initial rise of the electron density in air is at about 160 ns after the start of the pulse. The maximum $n_e$ value seems to be in the same order. However, the high electron density lasts about 50 ns longer compared to the case of ambient argon. The mean electron energy in figure 8.5 is shown for the same condition. The maximum of the electron energy is lower than for the argon-argon case, around 2.7 eV, but consistent with the electron density a plateau-like behaviour of $\langle E_e \rangle$ is found between 160 and 250 ns.

8.3.3 Temporal and spatial development of the electron density

As the TS intensity is linear with the scattering events, i.e. linear with the electron density, we can plot the intensity, summed over the wavelength, along the 1D strip as a representation of the electron density profile. In figure 8.7, 11 consecutive time frames, each shifted by 5 ns with an exposure time of 2 ns, are linearly interpolated in time and plotted into one image. As the streamer velocity is relatively constant and by assuming that the shape of the streamer head does not significantly change during the 50 ns time frame, we can convert the temporal axis to a spatial one, shown on the right side of the figure. Consequently, figure 8.7 presents a 2D spatial image of the electron density of the streamer head.

The overall shape of the streamer reminds of a "mushroom". A spherical shell of a high-energetic and dense electron distribution is preceding the streamer channel. This shape extends up to a diameter (FWHM) of 1.5 mm which is much broader than the actual channel. The plasma head decays evenly, although in the central region we find the onset of the actual streamer channel which exhibits a slower electron density decay.
Figure 8.7: Contour plot of Thomson scattering intensity as function of time and space. The TS signal is measured 3 mm away from the glass tube with a 2 ns exposure. Values below $10^{18}$ m$^{-3}$ do not correspond to densities as such low TS signals are related mostly to noise.

8.4 Discussion

The discussion section is organized in three parts based on the TS results presented in the previous sections. The first section deals with the interpretation of the measured electron energy. In the second section the streamer head and the electron density decay, leading to the formation of the streamer channel, is analysed. The last section discusses the shape of the electron density distribution in the streamer head and its discrepancy with the optical emission distribution.

8.4.1 The mean energy and the electron energy distribution function

The TS intensity as function of the squared wavelength shift corresponds to an electron energy distribution function (EEDF), because different electron velocities result in different spectral shifts of the scattered photons. The TS signal for all our measurements can be fitted very well to a Gaussian function. Consequently, we can assume that the observed electrons which are in the energy range of 0.15 – 7 eV obey a Maxwellian distribution. A few examples of the EEDFs from different time steps of the streamers with different plasma parameters are given in figure 8.8.

However, this does not imply that the whole EEDF is Maxwellian shaped. It was shown in [31] that for instance a depleted tail of a non-Maxwellian distribution has only a small influence on the apparent observed low energy part of the EEDF.
8.4: Discussion

simply due to the projection of the electron velocity distribution onto the ICCD. Therefore, such a depletion cannot easily be distinguished unless the fast electrons are observed directly.

The recorded TS spectra show no sign of asymmetry. Consequently, the electrons at the low energy part of the distribution are isotropic. It is expected that only in the high field regions the EEDF will be non-isotropic and under these conditions the Thomson scattering results should be considered with care, however, as mentioned above we did not observe signs of an anisotropic nature of the EEDF.

Nonetheless, the EEDF might be very depleted, as a simulation by BOLSIG+ [35] for an argon system, containing only electron momentum, electron excitation to Ar(4s) and electron ionization collisions, shows. In figure 8.9 the results of such simulations for different electric field strengths are shown. A large change in field strength is accompanied with a relatively small change of the mean energy from $2.2$ to $5.1$ eV. These results are to illustrate that the bulk of the electrons with energies below $E < 10$ eV is quite well described by a Maxwellian distribution while the tail is certainly not. The part of the EEDF which we cannot measure changes for instance the excitation of Ar states, which is strongly affected by the high energy tail of the EEDF.

8.4.2 Formation of the streamer channel

A drop in the electron density is found from the streamer head towards the channel on a time scale of tens of ns. However, the time scale of the formation of $\text{Ar}_2^+$ and

Figure 8.8: EEDFs obtained by TS from different time steps of an argon streamer in argon at 3 mm from the tube. Time points are (top to bottom plots) 160 ns, 150 ns and 500 ns.
dissociative recombination of the dimer ion can be estimated to be much larger. Even in the case that the dominant ion is $\text{Ar}_2^+$ (for cold atmospheric argon plasmas a reasonable assumption) and a rate of $k_{DR} = 8 \times 10^{-13} (300/T_e (\text{K}))^{0.64} \text{ m}^3 \text{s}^{-1}$ [36] we find with $n_e = 5 \times 10^{19} \text{ m}^{-3}$ and $T_e = 0.5 - 3 \text{ eV}$, decay times of $\tau_{DR} = 160 - 520 \text{ ns}$. These time scales can be indeed found after the voltage pulse, when the streamer channel is established. Three body electron-ion recombination has been found by Yu, Babaeva and Naidis [37] to cause a fast reduction in $n_e$ for streamers in liquid xenon where the recombination rates are strongly enhanced due to the significant higher electron densities. That process causes an electron density decay profile in the channel which reminds on the decay profile in figure 8.3, although the corresponding rate in our system with the relatively high $\langle E_e \rangle$ and $n_e$ below $10^{20} \text{ m}^{-3}$ is too small to explain the decrease of $n_e$. A possible explanation seems to be electron transport due to the electrical field in the ionization front. An estimation of the electron transport time scale $\tau_e$ due to the mobility in an electrical field $E$ can be made by $\tau_e = \nu_e^{-1} = rm_e\nu_c/(qE)$, where the $r = 0.5 \text{ mm}$ is the radius of the streamer head. An estimation of the electrical field strength of about $E = 10^9 \text{ Vm}^{-1}$ [20] and the collision frequency $\nu_c = 10^{12} \text{ Hz}$ (at $T_e = 2 \text{ eV}$) [38] results in $\tau_e \approx 3 \text{ ns}$ which is actually faster than the observed initial decay of $n_e$ that was about $15 \text{ ns}$. Nonetheless, detailed 2D models are necessary to investigate this more precisely.

We need to stress here the fact that the described streamer is operated at very low voltages. In fact a 3.5 kV pulse would not create in every pulse a streamer when no argon gas channel with pre-ionization is present. Not having huge over-voltages has the consequence that the propagation velocity is much slower than in typical corona streamers [4] and consequently much longer time scales are present for
recombination processes in the transition from the streamer head to the streamer channel.

In addition the mean electron energy in the streamer channel drops significantly down to a value of about 0.3 eV. In models of streamers in air also a significant reduction of the $E/N$ in the channel is observed and the remaining mean electron energy is only sufficient to balance the electron losses by some ionization. In the case of argon, a high density of Ar metastables will be created in recombination processes of molecular ions which can readily be re-ionized and require only a small mean electron energy [32, 39].

The plateau of $n_e$ and $\langle E_e \rangle$ found for the case of the Ar jet in ambient air must be related to significant excitation of nitrogen and oxygen. Especially nitrogen provides a large reservoir of possibilities to enhance or prolong ionization reactions. For instance $N_2(A)$ metastables are not quenched significantly by ground state $N_2$ and lower the ionization potential, but also super-elastic energy transfer from vibrationally excited nitrogen can maintain the bulk of the EEDF for quite some time at a constant level without the presence of an electric field [40].

### 8.4.3 The streamer head

In most streamer models, which are mainly in air, the production of the excited species corresponds more or less with the electron density and energy profile [41]. Due to the repetitive nature of the streamer in the jet, the charge and metastable density in the streamer channel is much higher compared to the surrounding background (see chapter 7) and thus the streamer remains "confined".

![Figure 8.10: Simulated evolution of the radial profile of the electron density for the time span of 200 µs, which corresponds to the time period between the voltage pulses. The model is taken from chapter 7 with the initial conditions taken at about 20 ns after the maximum of the streamer.](image)
In figure 8.10 we plot the calculated electron density at the start of the following voltage pulse ($t = 200\,\mu s$). The same model as in chapter 7 is used with a starting distribution of the electron density at $t = 0$ with a peak value of $n_e = 3 \times 10^{19}\,m^{-3}$ and a FWHM of 0.5 mm. The result shows a radial profile of the pre-ionization with a FWHM of 1.8 mm that corresponds very well with the measured diameter of the streamer head. That suggests that the width of the streamer is determined by this pre-ionization profile. The width of the ionization channel in figure 8.10 is found to be insensitive to the Ar metastable density or $Ar^+ / Ar_3^+$ ion fraction, as the afterglow is ruled by local recombination.

In our measurements the continuum emission and also in chapter 7 the plasma line emission is confined to the streamer channel with a FWHM of < 0.5 mm, which is roughly in agreement with reported streamer channel widths. The light emission originating only from the streamer channel is in contrast to the observed high electron densities and especially electron energies at the outer rim of the "mushroom". The lack of emitted light from the outer regions is thus remarkable. A lack of emission makes ionization by electron impact unlikely and indicates a stronger depletion of the high energy tail of the EEDF, off-axis compared to on-axis. Unfortunately such a depletion cannot be observed in our TS measurements.

If the broad streamer head is not only created by electron impact ionization, the question remains how the electrons are produced. Two other mechanisms for ionization are possible: photo-ionization and photo-detachment. Argon excimers ($E_{Ar^2} \approx 9.8\,eV$) or argon resonant radiation ($E_{1s5} \approx 11.6\,eV$) can ionize argon metastables or impurities. Especially excimer radiation, which is produced very efficiently at atmospheric pressure, has a large mean free path length, because of the lack of self absorption and could extend the ionization radius significantly compared to what is typically obtained from e.g. photo-ionization models or measurements in air [41, 42].

The impurity level in our setup can be estimated by residual Raman scattering signals to be at most 2\% air. However, the $O_2$ ground state cannot be directly ionized by the excimer radiation and the shape of the streamer head seems not to be significantly affected by different air impurity levels. It is also unclear whether the impurities can cause an ionization corresponding to an electron density of up to $10^{20}\,m^{-3}$.

Another option is photo-detachment. The $O_2$ impurities could easily lead to a significant amount of negative ions, although their density will be substantially less compared to the maximum electron density during the pulse and could only cause some additional initial electron density. An additional ionization mechanism will thus be necessary in this case.

It also needs to be mentioned that dust is present in the vessel. We observed that apparently the jet produces dust particles over time, which is observable by the stray light in the laser beam. As the needle electrode is at a positive voltage, this might be caused by sputtering of the quartz tube. These dust particles might be
charged from a previous streamer and larger amounts of electrons can be photo-detached by plasma UV emission and contribute to the ionization.

Both proposed mechanisms cannot fully explain the discrepancy between $n_e$ and emission profiles as it will depend on species distributions and the production of excited species which cause the radiation and their effective lifetime. In case of photo-ionization one might expect an electron density profile peaking at the centre [9] and by photo-detachment a more ring-like profile, as more impurities are outside the jet. Most likely a combination of processes takes place.

8.5 Conclusion

In this work, for the first time the temporally and spatially resolved electron densities and energies are reported of a guided argon streamer in ambient argon and air. A strong initial $n_e$-overshoot with a maximum of $7 \cdot 10^{19}$ m$^{-3}$ and a mean electron energy of 4.5 eV, followed by a steep decay is found. Strong differences between the electron density profile and the emission profile are observed. Some suggestion are given for a possible mechanism.
References


Chapter 9

Cell proliferation and bacteria inactivation induced by a ns pulsed CAPPJ *

Abstract

A ns pulsed atmospheric pressure helium plasma jet was used to treat prokaryotic (Pseudomonas aeruginosa) and eukaryotic cells (CMT 93 and Caco-2). To characterize the plasma jet, the gas phase concentration of ozone and relative fluxes of ions have been measured with a molecular beam mass spectrometer. These measurements have been complemented with measurements of the hydrogen peroxide concentrations and the pH in the liquid phase. The results suggest the importance of O$_3$, H$_2$O$_2$ and ions. A parameter window to inactivate bacteria at safe levels for eukaryotic cells has been obtained, similar to chapter 6. Furthermore a real time cell analyser has been used in order to investigate the effect of the ns pulsed plasma jet on the proliferation of eukaryotic cells.

*This work is partly performed at the Tytgat Institut at the AMC Amsterdam and at the Burn Wound Centre in Beverwijk in collaboration with: F.W.M Hilbers, B.K.H.L. Boukema, J. Stap, M. Lowenberg, W.J. de Jonge and P.J. Bruggeman.
Chapter 9: Cell proliferation and bacteria inactivation by ns pulsed CAPPI

9.1 Introduction

As introduced in the previous chapters, plasma jets can also be produced by monopolar nanosecond (ns) voltage pulses. In this case guided streamers are produced. The main difference of the nanosecond pulsed plasmas which are driven at a kHz repetition rate, over the RF excited plasma jets, is that due to the very short voltage pulses, a plasma is only created for durations smaller than 1% of the pulse period. This is also the main reason why the light emission of the guided streamers is usually lower than for RF discharges. In addition, as estimated in chapter 7 a 5 kV results in a maximum energy of 50 µJ, this results in an average power of 0.25 W, which is smaller than the plasma power for typical RF plasma jets (see previous chapters).

Due to the very low duty cycle and average dissipated power, the gas temperature of the guided streamers is in most cases not significantly exceeding room temperature [1]. Naidis has simulated a guided streamer operated with helium with water vapour mixtures and found peak electron densities in the order of $10^{18} \text{m}^{-3}$ and OH densities in the order of $10^{19} \text{m}^{-3}$. Compared to typical RF jets these densities are at least one order of magnitude smaller than found in RF jets for which $n_e$ in the order of $10^{20} \text{m}^{-3}$ and $(n_{\text{OH}}$ in the order of $10^{21} \text{m}^{-3}$) have been found [2, 3]. The low gas temperature and the lower reactive species and electron density makes the kHz plasma sources ideal for "low plasma dose" experiments such as "cell parameter modification" without damaging the cells. A smaller sterilization capacity of low frequency plasmas compared to high frequency plasmas (at the same input power) has been also found by Seo et al. [1].

In this work a nanosecond kHz pulsed helium plasma jet has been used in order to find treatment conditions for bacteria inactivation and eukaryotic cell proliferation. It is shown that the plasma treatment is selective for prokaryotic and eukaryotic cells, depending on the medium the cells are suspended in. In addition, in view of the works by Kalghatgi et al. [4, 5] , Tipa et al. [6] who reported enhanced cell proliferation after plasma treatment, a detailed statistical analysis of time resolved cell index measurements is performed to assess cell proliferation for the plasma device and the experimental conditions of the performed study.

The chapter is outlined as follows. In section 9.2 the experimental setup, the diagnostics and the procedures are introduced. The physical and chemical properties of the plasma jet are presented in section 9.3. In section 9.5 the results of the bacteria inactivation and the cell proliferation are shown and discussed. We summarise and conclude in section 9.6.
9.2 Experimental setup and procedure

For the experiments reported in this chapter a kHz nanosecond positively pulsed high voltage plasma jet is used as described in chapter 7. The main difference is that in the previous chapters argon is mostly used as a carrier gas, while in this chapter results on the helium jet are reported. A schematic of the setup is shown in chapter 7 in figure 7.1 (for the work presented in this chapter the vacuum chamber is removed from the setup). The applied voltage is 4 kV, if not stated otherwise, the pulse length is 500 ns and the pulse frequency is 5 kHz. A helium flow of 1 slm is used. A CCD-camera (Sony DSC-R1) has been used to obtain time averaged images of the plasma jet. Time resolved images are obtained with an ICCD camera (Stanford Computer Optics 4Picos) and synchronised with the kHz pulse of the applied voltage. A lens with a focus of 30 cm is placed between the ICCD camera and the plasma which images, together with a photographic lens, the plasma on the ICCD.

Voltage and current waveforms are obtained with a high voltage probe (Tektronix P6015A) and with a current monitor (Pearson 6656) which are read out by an oscilloscope (Agilent Technologies DSO 1024A, 200 MHz, 2 GSa/s). The molecular beam mass spectrometer is used in the same configuration as described in chapter 4 and the $\text{H}_2\text{O}_2$ concentration measurements in the liquid have been performed with the same method and devices as described in chapter 5. The pH measurements have been performed with a portable pH meter (WTW, Multi 340i).

For the experiments on cells the CMT 93 cell line is used (either 10000 or 15000 cells per well) or Caco-2 cells (15000 cells per well), for the treatment on bacteria $P. \text{aeruginosa}$ (strain PAO1) is used (10$^7$ CFU/ml). During the treatment the bacteria and the cells where suspended in 150 $\mu$l of Dulbecco Modified Eagle Medium (DMEM with 4.5 g/L Glucose, L-Glutamine and Pyruvate (Invitrogen)) or saline. $P. \text{aeruginosa}$ was routinely cultured on Luria Broth (LB, Invitrogen, Paisley, UK) agar at 37°C. Bacteria from a logarithmic culture in 5 ml LB were diluted in 0.85 % NaCl, to approximately 10$^7$ colony forming units (CFU)/ml, based on $\text{OD}_{650}$ (optical density measurements at 650 nm). To quantify the CFU/ml before and after treatment, bacterial suspensions were serially diluted and plated on LB agar plates. The antibacterial effect of the treatment was calculated by the log reduction log($N_T/N_C$), where $N_T$ is the number of viable cells after treatment and $N_C$ the number of viable cells in control samples.

To investigate the behaviour of the eukaryotic cells, the cells have been suspended in DMEM and the cell index has been monitored inside the incubator in a real time cell analyser (RTCA) (xCELLigence Roche) [7–9] and at the end of the measurement an MTT assay has been performed to measure the cell activity (see chapter 6 for details). The real time cell analyser uses well plates with golden electrodes at the bottom of the well. The golden electrodes are needed to measure
the electrical resistance. The cell index, \( C_I \), is defined by, \( C_I = \frac{Z_i - Z_0}{15 \Omega} \), with \( Z_i \) being the measured resistance during the monitoring and \( Z_0 \) being the initial resistance at the beginning of the measurements.

The resistance of the system can increase by an increase of the cell number on the golden bottom or by an increase of the cell surface attached to the golden bottom. A decrease is observed by rounded up cells and/or dead and detached cells [7]. The plasma treatment on bacteria and cells is performed at a distance of 13 mm between the surface of the liquid and the end of the glass tube of the plasma source, if not stated otherwise. In this configuration the plasma is in direct contact with the water surface. After one day of incubation and monitoring, the cells are treated by the plasma. After plasma treatment the cells are placed back into the cell analyser and are monitored continuously for one to three more days.

For the experiments with saline solution shown in this work, the bacteria have been kept in the medium 2 minutes after treatment (i.e. 2 min incubation time), afterwards the solution and the bacteria are diluted and plated on agar plates. Also the CMT 93 cells were kept in the saline for an incubation time of 2 min. The medium was replaced 2 min after plasma treatment by fresh DMEM and the plates were placed back into the xCELLigence. The maximum total time that the cells were in the saline solution was 11.5 min.

For the experiments with DMEM the cells have been treated and the solution has not been replaced after the treatment. For \( P. \) aeruginosa the incubation time after treatment in the treated solution has been varied from seconds to up to 5 hours, before dilution and plating was performed.

**Comparison MTT assay with real time cell analyser**

As MTT assays yield information on the cell activity and the RTCA yields only combined information on detachment and cell damage, both of these measurements give a different and only an indirect information on cell death. It is thus very useful to compare both methods.

Figure 9.1 shows an example of the results of the real time cell analyser (xCELLigence). For this graph Caco-2 cells (15000 cells per well) have been used and treated with a plasma jet (4 kV, 500 ns, 1 slm helium) at a distance of 13 mm between the end of the glass tube and the liquid surface. In this configuration the plasma is directly in contact with the water surface. The cells are suspended in DMEM and the DMEM is not replaced after treatment. Twenty hours after treatment (i.e. forty hours after start of the RTCA measurement) the viability of the cells has been checked with MTT assays and compared with the results of the cell index measurement obtained by the xCELLigence (table 9.1).

The cell index curves show a clear dependency in treatment time and voltage of the plasma, i.e. longer treatment time (longer than 2 min) and higher voltage
9.2: Experimental setup and procedure

Figure 9.1: Comparison of the xCelligence measurement and an MTT assay from Caco-2 cells treated with a helium plasma at a distance of 13 mm between the end of glass tube and surface of the DMEM. The amount of visible data points have been reduced to 42 points per curve and the error bars to 5 points per curve, for better visibility.

(4 kV) lead to a strong reduction of the cell index. The curve of the cell index indicates that the cells are either detached from the gold surface or rounded up, which could indicate cell death. This is also visible in the MTT assay results, which shows the lowest percentage of cell viability for the 4 kV and 2 min and 3 min treatment as can be seen in table 9.1.

The cells in the control plate, which was out of the incubator, the gas only control and the other plasma treated cells, show a typical cell growth curve with a growth phase in the first 20 hours and a stationary phase afterwards. When the cells are out of the incubator a drop in cell index is visible, possibly because the cells are in a resting phase. This is caused by the exposure of the cell to a non-ideal environment, i.e. open air at 20°. Since the cells are only in a resting phase, growth starts after the well plates have been placed back into the incubator. The advantage of the real time cell analyser is clearly the time resolved measurement of the cell index. It provides a more in depth view of the cell behaviour after plasma treatment, while the MTT assay is only a measurement of the cell activity at one specific time point and has a larger accuracy for the current conditions.

The two methods give comparable results for the 4 kV and longer than 2 min treatment time treatment, showing either a lowering of the cell index or a lower percentage of active cells, respectively. However, for the other conditions it can be seen that results of the MTT assay show a discrepancy compared to the results...
Table 9.1: Comparison between the cell index at 43 h after the start of the RTCA measurement with MTT assay performed directly after the end of the RTCA measurement (i.e. 43 h). Each value is the results of the analysis of two wells. The error is the standard deviation of the two measurements.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CI [a.u.]</th>
<th>MTT [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>gas only, 3 min</td>
<td>5.4 ± 0.4</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>untreated, out of incubator</td>
<td>5.6 ± 0.1</td>
<td>77 ± 6</td>
</tr>
<tr>
<td>4 kV, 1 min</td>
<td>6.4 ± 0.3</td>
<td>88 ± 1</td>
</tr>
<tr>
<td>4 kV, 30 s</td>
<td>6.4 ± 0.7</td>
<td>118 ± 1</td>
</tr>
<tr>
<td>3 kV, 3 min</td>
<td>5.5 ± 1.3</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>4 kV, 3 min</td>
<td>0.45 ± 0.05</td>
<td>60 ± 40</td>
</tr>
<tr>
<td>4 kV, 2 min</td>
<td>2.1 ± 0.3</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>untreated, out of incubator</td>
<td>4.9 ± 0.5</td>
<td>90 ± 20</td>
</tr>
</tbody>
</table>

obtained by the RTCA. For example, the MTT assay indicates that the treatment of 4 kV and 30 s leads to the highest cell activity, indicating a positive influence of the plasma treatment. However, the cell index curve shows that for this condition the cell index was higher before the plasma treatment. Fluctuations between different wells could thus influence the results significantly if no proper reference is taken with the MTT assay. As the RTCA (xCelligence) yields more information, in the remainder of this work, the RTCA method is used to characterise the response of the cells after plasma treatment.

Figure 9.2 shows the cell index as a function of time for the untreated control cells which did not leave the incubator. This figure clearly shows the large variation of the cell cycle on the different wells. As the change of the cell index is relatively small compared to the well-to-well fluctuations, it is important to perform enough measurements to allow us to analyse the results taking into consideration the proper statistics.
Plasma characteristics

Figure 9.3 shows an example of the plasma used at an applied voltage of 3 kV with a tap water substrate (not grounded) at different distances between the nozzle and the water surface. As the plasma is in direct contact with the water surface a cathode layer is formed on top of the water surface as is clearly observed in figure 9.3(b). Figure 9.3(c) illustrates that with decreasing the distance between jet and liquid the plasma intensity increases. To obtain a better insight into the morphology of the plasma in contact with a water substrate, nanosecond time resolved ICCD images have been obtained for a non-touching and touching plasma.

In figure 9.4(a) a water surface is 12 mm away from the plasma (driven at 2 kV) and the plasma is not in direct contact with the liquid. Since the plasma is not influenced by the water surface, it behaves like the helium plasma bullets investigated thoroughly in various research groups (see [10] and references therein). The streamer propagates out of the glass tube and the propagation stops when the voltage is switched off. This event is followed by a re-emission at the needle tip, as also observed by Karakas et al [11]. The current and voltage waveforms
Figure 9.3: Images of the plasma jet used in this work. The exposure time of the images and the distance of the water (tap water, not grounded) are indicated in the images. (a) 3 kV, plasma without substrate. (b) 3 kV, plasma in contact with water surface. (c) 3 kV, plasma in contact with water surface.

Figure 9.5: Images of the plasma jet used in this work. The exposure time of the images and the distance of the water (tap water, not grounded) are indicated in the images. (a) 3 kV, plasma without substrate. (b) 3 kV, plasma in contact with water surface. (c) 3 kV, plasma in contact with water surface.

are shown in figure 9.5. As shown in chapter 7 the current is mainly capacitive.

By increasing the voltage, the length of the visible plasma increases until it reaches the liquid electrode, as shown in figure 9.4(b). The streamer reaches the electrode at around 120 ns after the start of the pulse. Due to the higher voltage and the water at its vicinity, the velocity of the streamer is increased (see chapter 3 for similar effects with the RF plasma jet).

The emission induced by the streamer vanishes when the streamer reaches the water surface and only emission at the needle is observed. This is due to the drop in the E-field in the bulk of the gap when the streamer reaches the second electrode. Approximately 40 ns after the streamer reached the water, emission is visible at the water surface indicating that a sheath region is formed (cathode region), while the light close to the glass tube decreases. A similar behaviour is also reported in [12, 13]. This phenomena is consistent with the formation of a glow discharge which has the highest emission intensity in the cathode sheath region (negative glow).

Figure 9.6 shows an emission spectrum of the $N_2$(C-B)(0,0)-band and a simulated spectrum obtained by Specair [14]. The best fit corresponds with a rotational
Figure 9.4: Nanosecond time resolved images of (a) a 2.0 kV plasma not in contact with a water surface (b) a 3.2 kV plasma in contact with a water surface. The water surface was in both cases 12 mm away from the nozzle. The exposure time was in both cases 10 ns. The voltage gain of the multiplier and the number of accumulations was for (a) 220 V and 15000 and for (b) 100 V and 7500, respectively.
temperature of $340 \pm 30$ K. As shown in chapter 2 the rotational temperature of $N_2$(C-B) is a good representation of the gas temperature in a helium plasma. The emission spectrum is recorded for a 2 kV helium plasma in contact with water at 1.5 mm above the water surface. The emission has been also obtained spatially resolved and for the same plasma settings operating without a substrate but no significant difference in gas temperature has been found. This indicates that the plasma remains close to room temperature and that heating even when a glow discharge is formed is, if occurring, small. However, it has to be noted that the highest emission originates from the bullet phase, rather than the glow phase of the discharge, which could indicate that the rotational temperature is only representative for the gas temperature in the bullet phase.

The ozone concentration has also been measured for the plasma bullet for three different distances (either 13, 18 and 23 mm or 15, 18 and 23 mm) for two different voltages (either 2.5 or 4 kV, respectively) and is plotted in figure 9.7 with a MBMS as described in chapter 4 (i.e. well attached to the metal sample plate, suction ring mounted on the MBMS inlet). For the 4 kV case the plasma was always in direct contact with the metal orifice at the chosen distances. For a voltage of 2.5 kV the plasma was not in direct contact at distances larger than 18 mm. In both cases it can be seen that the ozone concentration does not depend on the distance from the jet to the orifice. Note that at 13 mm and 4 kV the jet produces a spark between the nozzle and the orifice of the MBMS and therefore no measurement is included for this condition in figure 9.7.

The $O_3$ calibration has been performed in a pure helium atmosphere while the plasma jet was open to air during the measurements. Mixing of air results in a different supersonic expansion and therefore in different species densities in the
9.3: Plasma characteristics

Figure 9.6: Measured and simulated rotational spectrum of $N_2$(C-B)(0,0) with a best fit corresponding to a rotational temperature of $340 \pm 30$ K. The spectrum is recorded 1.5 mm away from the water surface for a 2 kV helium plasma which was in direct contact with the water surface.

molecular beam in the second pumping stage which can lead to an underestimate of the signal up to a factor of 4, compared to measurements performed in pure helium [15]. However simulations of the mole fraction of nitrogen in a similar helium plasma jet, have shown that the mole fraction of nitrogen is at a distance of 50 mm well below 30 % [16], which leads in the current experiment to a maximum error of 40 %.

With a measured ozone concentration of $7 \cdot 10^{20}$ m$^{-3}$, using Henry’s law and a flow of 1 slm into a volume of 150 µl, a production rate of ozone $R_{O_3}$ of 34 µM/s in the liquid is calculated.

$$c_{O_3} = \frac{n_{O_3}}{n_0} \cdot p_0 \cdot k_H \cdot Q \cdot \frac{1}{V},$$

with $n_{O_3}$ being the density of ozone and $n_0 = 2.5 \cdot 10^{25}$ in the gas phase in m$^{-3}$, $Q$ the flow in l/s, $k_H$ Henry’s constant for ozone in mol/(l·bar), $p = 1$ bar and $V$ the volume in l.

This is a factor 12 higher than the concentration expected for the treatment of the RF jet and in the range where bactericidal effect of ozone are reported (see table 5.3 in chapter 5). The transfer of $O_3$ into the liquid will be larger as the jet introduces a mixing into the liquid. Without consideration of Henry’s law constant
an even 4 times larger concentration is found in the liquid. These calculations assume that ozone is not significantly reduced by reactions in the liquid phase such as the reaction with NO$_2^−$. The NO density in the jet effluent was below the detection limit of the MBMS (see also further).

Figure 9.8 shows the fluxes of positive ions to a substrate measured by the MBMS. Since a positive mono-polar voltage pulse has been used, negative ions are expected not to play a dominant role for the investigated conditions. Although, it has to be noted, that since in both cases (i.e. treatment of liquid and MBMS measurements), the plasma is in contact with the liquid and metal substrates, results obtained by the mass spectrometer have to be extrapolated to the biological measurement conditions with care due to the difference in electrical properties of the substrates (see also chapter 3 for the RF jet).

When the jet is mounted to the MBMS, non-touching conditions are established at distances longer than 39 mm between nozzle and metal orifice. As can be seen in figure 9.8, the ion signal drops the further the plasma is away from the metal orifice being below the detection limit, when the jet is no longer touching. The dominant ion is N$_2^+$, which is to be expected due to significant air mixing at the measured position. At the closest distance between nozzle and orifice used, also helium ions were obtained. A closer distance could not be used due to arcing. Opposed to the results in chapter 5, no significant amount of large water cluster ions have been detected. This is most likely related to the fact that ions are sampled in the active plasma zone and not in the far effluent as in chapter 5.
In this section the hydrogen peroxide concentration and the pH are reported and analysed to assess the plasma induced chemistry.

Figure 9.9 shows the pH value for a treatment of 2 ml distilled water for 2 different distances as a function of the treatment time. The treatment time is longer than the 90 s treatment time used for the bacteria, due to the larger volume of the liquid and the assumption that the treatment time is proportional to the volume of the liquid. Note as well that the visible plasma plume was clearly not in contact any more for distances larger or equal than 41 mm. In the smaller well in which the bacteria are treated, the induced turbulences inside the well reduce the length of the visible plasma plume at small distances. The results obtained for the pH thus only allow us to draw qualitative but no quantitative conclusions.

The nitrite and nitrate concentrations in distilled water as a function of treatment time have been obtained with ion chromatography as described in chapter 5. The treated volume was 2 ml, as with the pH measurements. The results of these measurements are shown in figure 9.10.

As the main ion is $N_2^+$ (see figure 9.8) the following reactions at the water interface will occur:
Figure 9.9: pH for two different distances between glass tube and the surface of the liquid as a function of time. A liquid volume of 2ml volume of distilled water was treated with a 4kV, 5kHz, 500ns pulse plasma.

\[
\begin{align*}
\text{N}_2^+ + \text{H}_2\text{O} & \rightarrow \text{H}_3\text{O}^+ + \text{N}_2 \quad (9.2) \\
\text{H}_2\text{O}^+ + \text{H}_2\text{O} & \rightarrow \text{H}_3\text{O}^+ + \text{OH} \quad (9.3)
\end{align*}
\]

This causes acidification of the liquid and a larger production of OH. It is thus to be expected that the main species in the liquid are H$_2$O$_2$, O$_3$ and NO$_x$. NO and NO$_2^-$ in the liquid phase have been also shown for a similar jet to be present at relatively high concentrations [18].

It can be seen that a higher nitrite and nitrate concentration is measured when the plasma is close and in direct contact with the liquid surface. Comparing these results with figure 5.10, the kHz pulse plasma in contact with the liquid surface produced, for the same treatment time, slightly higher NO$_x^-$ concentrations than measured with the argon RF plasma jet not in contact with the liquid surface. Note that the volume of the treated liquid in figure 5.10 was higher (3.35ml) than in this chapter (2ml).

Assuming that NO$_x^-$ are the dominant negative ions in the liquid, the NO$_x^-$ concentration should correspond to the pH [17]. Table 9.2 shows the estimated sum of the concentration of nitrite and nitrate during the pH treatment. Comparing these results, when the plasma is in direct contact with the liquid surface (i.e.
13 mm) the calculated $\text{NO}_x^-$ concentration obtained from the pH is lower than the measured concentration. The acidification is thus not only due to the formation of NO, HNO$_2$ and HNO$_3$ by the plasma.

Note that, Ikawa et al. have used a high voltage (-3.5 to +5 kV), 13.9 kHz driven helium plasma jet in direct contact with distilled water. In this case, the drop in pH (in a 100 µl liquid) reached values of 4.2 for the longest treatment time (300 s). This value and following from that, the $\text{NO}_x^-$ concentration in the liquid is similar to the values obtained in this work [17]. No non-touching conditions have been reported in [17].

An increase of the $\text{NO}_x^-$ concentration at decreasing distances is remarkable, as one would expect that $\text{NO}_x^-$ does not depend strongly on the distance since the NO or at least the combined NO, NO$_2$, NO$_3$, HNO$_2$ and HNO$_3$ in the gas phase will not decrease significantly with distance. However, NO production requires elevated temperatures. An increase of temperature was not measured when the plasma was in direct contact with the liquid surface, however, as discussed above, the time average measured gas temperature could be only an image of the intense short lived plasma bullet, rather than the gas temperature when the jet is connecting to the liquid surface.

Apart from the pH and $\text{NO}_x^-$, $\text{H}_2\text{O}_2$ is also measured for three different treatment times (5, 60 and 120 s) and two different distances (13 and 19.5 mm). These measurements were performed at the same conditions as the treatment with the
biological samples, i.e. 150µl saline solution in xCELLigence well plate, with a 4 kV, 5 kHz, 500 ns pulse plasma. A clear correspondence is visible both in treatment time and treatment distance, i.e. longer treatment times and shorter distances results in the highest measured concentration of H$_2$O$_2$ which is in the same range as seen by the argon RF plasma in chapter 5. By increasing the distance between nozzle and liquid, the hydrogen peroxide concentration drops until it is below the detection limit (i.e. below 100 µM), when the jet is no longer in direct contact with the liquid surface.

In addition, as mentioned in chapter 3, the density of excited and/or ionised species could increase in the "touching-mode", due to an increase in plasma power and/or gas temperature.
9.5 Bacteria inactivation and cell proliferation

In this section the effects of the ns pulsed plasma jet on bacteria and cells is investigated further.

9.5.1 Treatment of P. aeruginosa in saline solution

Tests have been performed at the start of the measurement campaign by treating the bacteria with the plasma in saline and leaving the bacteria in the treated solution for various time intervals. It was found that no significant log reduction is measured when the bacteria are immediately plated after treatment. However, leaving the bacteria longer (up to 10 minutes) in the treated saline solution can lead to a complete inactivation corresponding to a log reduction of 7, depending on treatment and incubation time.

Table 9.3 shows the logarithmic reduction of P. aeruginosa treated in saline solution for 4 different incubation times. One plasma setting (4 kV, 5 kHz, 500 ns) has been used and the treatment time and distance setting was 90 s and 13 mm, respectively. It shows that more bacteria are inactivated when left longer in the treated medium. This indicates a delay of response of the bacteria to the treatment, in contrary to the results seen in chapter 5, where a significant log reduction
Table 9.3: Log reduction for different incubation times. The plasma settings correspond to 4 kV, 5 kHz, 500 ns pulse width. The distance from the nozzle to the liquid surface was 13 mm and the treatment time was 90 s. * indicates that the measurement is performed 3 times. ** indicates that the measurement is performed 8 times. *** indicates that the measurement is performed once.

<table>
<thead>
<tr>
<th>Incubation time [s]</th>
<th>Log reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>1.28 ± 0.14</td>
</tr>
<tr>
<td>120**</td>
<td>2.8 ± 1.0</td>
</tr>
<tr>
<td>300***</td>
<td>2.9</td>
</tr>
<tr>
<td>600***</td>
<td>5.4</td>
</tr>
</tbody>
</table>

was observed immediately after treatment. All the other experiments have been obtained with an incubation time of 2 min after the end of the treatment, if not indicated otherwise.

Figure 9.12: Comparison of the RF argon plasma with the ns-pulsed helium plasma for *P. aeruginosa* treated in saline solution.

Figure 9.12 shows the log reduction induced by the helium plasma as a function of treatment time compared with the results obtained by the RF argon plasma jet used in chapter 6. With the choice of the proper conditions similar results in log reduction can be obtained for both discharges. Note that the bacteria treated with the RF argon plasma have been immediately diluted after treatment, while the bacteria with the helium plasma were left in the treated saline solution for a duration of two minutes, as mentioned above. Next to the difference in incubation time, the helium plasma was in direct contact with the liquid, while the tip of the visible plume of the argon plasma was 8 mm above the liquid surface.
To obtain a qualitative assessment between touching and non-touching effects on bacteria inactivation, figure 9.13 shows the log reduction of the bacteria as a function of distance between the nozzle and the liquid surface (leading to touching and non-touching conditions of the jet) is shown in figure 11. The plasma is found to be in direct contact with the water surface up to a distance of approximately 18 mm. The plasma is no longer in contact with the water surface approximately 25 m away from the substrate. In between these distances the plasma connects or disconnects randomly with the water surface. A transition point in bacteria inactivation can be seen, when the plasma is not in contact with the solution at 26 mm.

9.5.2 Treatment of CMT 93 cells in saline solution

Figure 9.14 shows the treatment performed with CMT 93 cells for the same conditions as the bacteria inactivation study reported in figure 9.12, i.e. the treatment is performed in saline solution and 2 minutes after treatment the saline solution was replaced with fresh DMEM. Three controls are shown, i.e. cells untreated and not removed from the incubator, cells untreated but removed from the incubator for a time identical as the plasma treated samples, cells untreated but suspended in saline for 5 minutes. In addition three treatments of different treatment times, i.e. 90, 120 and 180 s are shown. The distance between liquid surface and nozzle was in all cases 13 mm. It can be seen that for all cases, the cells continued
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Figure 9.14: CMT 93-cells in saline treated with a 4 kV, the distance between the nozzle and the liquid surface was 13 mm. Three different control wells are plotted as well. Every well contained $10^4$ cells. The number between the brackets represents the number of measurements performed for the indicated settings. A zoomed-in view is included to clearly illustrate the short time response of the cell index after treatment.

A drop in cell index is visible at the first measured time point after the treatment (i.e. 27 hours and 44 min after the start of the experiment), due to the stress the cells had by staying outside the incubator and suspending them into saline. However, the cells recover in all six cases when placed back in the incubator. Comparing the cell index in the first few hours after treatment, a slight increase is seen for the treated wells, which settles after 5 hours (see zoom-in view in figure 9.14).

The higher survivability of eukaryotic cells compared to bacteria is attributed in chapter 6 to the larger surface area of the cell, and due to the more sophisticated repair mechanisms against reactive oxygen and nitrogen species induced damage.

9.5.3 Treatment of *P. aeruginosa* and CMT 93 cells in DMEM

Treatment of *P. aeruginosa* has been also performed in DMEM as described above without changing the medium after the treatment. However no significant reduction of colony forming units have been observed for the used treatment and incubation times (up to 90 s and up to 5 hours, respectively).
Figure 9.15: Results of the cell index of plasma treatment of 15000 CMT 93-cells per well suspended in DMEM. The voltage was 4 kV and the distance between the nozzle and the liquid surface was 13 mm. Data points have been reduced to 34 points per curve and the number of error bars have been reduced for better visibility. Each setting has been performed for two wells.

The opposite effect is seen when the CMT 93 cells where treated for the same conditions. Figure 9.15 shows the outcome of the experiment. It can be seen that a decline in the cell index is visible for wells treated for treatment times longer than 45 s. For treatment times longer than 60 s the plasma treatment delivers a lethal dose as can be seen since the cells completely stopped growing and did not seem to recover from the treatment.

The reason that for the buffered and nutrient rich medium (DMEM) the bacteria survive lies probably in the fact that due to the buffer, the pH does not decrease as observed in the results presented in chapter 6. The CMT 93 cells in comparison, probably round up due to a higher concentration of hydrogen peroxide and possibly due to a reduction of the nutrients in the DMEM and the prolonged incubation time in this medium.

Treatments have also been performed with the DMEM replaced after a 2 min incubation time after plasma treatment and are presented in figure 9.16. When the DMEM is replaced after the treatment the cell index of the treated and untreated cells continues to increase, while the cell index of the cells in the non-refreshed medium decreases and becomes 0 after 5 hours. This result indicates that the plasma is changing the composition of the DMEM and/or that the increased concentration of plasma produced species is large enough to result in a stop of cell growth/attachment due to a non-ideal environment for the cells on time scales of more than 3 hours.
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Figure 9.16: Results of the cell index of plasma treatment of 15000 CMT 93-cells per well suspended in DMEM either with DMEM replaced 2 min after treatment (indicated with "new DMEM") or not (indicated with "no new DMEM"). The voltage was 4 kV and the distance between the nozzle and the liquid surface was 13 mm. Data points have been reduced to 40 points per curve and the number of error bars have been reduced for better visibility. Each setting has been performed for four wells.

9.5.4 Investigation of cell proliferation in DMEM

To investigate possible enhancement of cell proliferation induced by the plasma treatment, tests have been performed for CMT 93 cells in DMEM treated with a 4 kV plasma for less than 45 s, as can be seen in figure 9.15. The statistical analysis of the result is shown in 9.17. In this figure the ratio of the slope of the cell index for an interval up to five hours before and up to five hours after the treatment is shown for 2 treatment conditions and their respective control slopes of untreated wells.

Due to the high variation in slope of the cells index, the experiments have been performed between 25 to 36 times, as indicated in figure 9.17. In both cases the slopes of the treated wells show a slight difference compared to the control wells, however the error bars overlap, indicating not a significant change in cell proliferation due to plasma treatment, for the conditions used in this work. Also using the Mann-Whitney-U-test revealed no significant difference between control and treated cells.

The difference between the results obtained by us and the results on cell proliferation observed by Kalghatgi et al. and Tipa et al. might be due to the fact that
in these experiments the cells have been treated directly only with a relatively thin layer of liquid above the cells, while in our case 6.5 mm of (buffered) liquid medium was between the plasma and the cells, resulting in different species delivered to the cells. Additionally, the hydrogen peroxide in the liquid phase might cause an inhibition of the cell growth.

9.6 Conclusion

![Figure 9.17: Ratio of the slope of the cell index before and after treatment for two different treatments and control wells. The control wells indicated "control in" were left in the incubator during the whole experiment and the wells indicated "control out" were the wells which were left outside the incubator during the treatment of the other well plates. 15000 CMT 93-cells per well were suspended in DMEM. The voltage was 4 kV and the distance between the nozzle and the liquid surface was 13 mm.](image)

In this chapter a ns pulsed helium plasma jet has been used for treatment of prokaryotic and eukaryotic cells suspended in saline or DMEM. Gas phase measurements of the plasma have shown for the used treatment conditions, ozone concentrations up to 28 ppm. Hydrogen peroxide in the liquid phase was found up to the order of 2.1 mM, while the pH dropped down to 4.2. Ion chromatography measurements show that the pH is not determined by the NO\textsuperscript{−} concentration which indicates additional acidification possibly by ionic processes. The NO\textsuperscript{−} concentration was higher when the visible plasma plume was in direct contact with the liquid surface, which could originate from an increase in power and/or temporally increased heating.
Conditions have been observed where bacteria are inactivated while cells are not harmed for the same treatment conditions in saline solution. The opposite effect was observed when the cells and the bacteria were treated in DMEM without changing the medium. No bacteria inactivation was found, when the bacteria were treated in DMEM and left in the treated medium for up to 5 hours, while the CMT 93 cells were clearly damaged. The increased H$_2$O$_2$ leads most likely to inactivation of mammalian cells for long exposures, however, further study is necessary to reach a clear conclusion. Further investigations on cell proliferation showed no significant effect of plasma treatment on cell proliferation for the treatment conditions used here and showed the importance of statistical evaluation of the data due to large individual fluctuations in the data set. Comparing the ns pulsed jet with the RF jet we observed, that a significant log reduction of *P. aeruginosa* with the former one was only visible, when the plasma was in direct contact with the water surface, while for the RF jet also a non-contact mode, resulted in bacteria inactivation at similar treatment times. In addition, a significant bacteria inactivation was only observed for an incubation time after treatment of tens of seconds to minutes, while for the RF jet no long incubation time is needed. Although it seems that bacteria inactivation is still likely to be caused by the same mechanism and needs an increased NO$_3^-$ concentration (to reach similar concentrations as the RF jet) which is achieved during touching mode of the plasma jet.
References


Chapter 10

Conclusion

The goal of this work has been to investigate the physics and chemistry of cold atmospheric pressure plasma jets and their interaction with prokaryotic and eukaryotic cells. To this end, we built a plasma jet which can be operated in different modes, i.e. linear or cross-field, continuous or kHz modulated RF, and mono-polar nanosecond pulsed. The jet can be operated in helium or argon as carrier gas, with admixture of air, N\textsubscript{2} or O\textsubscript{2}, both in ambient air or in a helium or argon environment.

Many different diagnostics have been performed on this plasma source. We have used fast imaging, current and voltage measurements, optical emission spectroscopy, mass spectrometry and Thomson scattering. In addition, the jet has been characterized by co-workers using LIF, TALIF and UV absorption to obtain absolute densities of NO, O and O\textsubscript{2}. This provides very detailed information on the operation and properties of the jet. Furthermore, the jet is used to treat prokaryotic and eukaryotic cells to study the mechanisms of plasma-bio interactions. In the following 3 sections we present the key results and conclusions arising from these studies.

Plasma properties of the RF jet

We developed a simple method to measure the power dissipated in the plasma. By integrating the measured voltage and current of the electrical circuit the power dissipated in the plasma and matching box is obtained. It is shown that the power dissipated in the matching network can be obtained by measuring the power at the same root mean square current when the plasma is switched off and can thus be used to obtain the plasma dissipated power. We have shown that with this
technique also the time resolved power dissipation of kHz modulated RF plasmas can be measured.

The gas temperature is obtained by OES in particular by the rotational spectrum of molecules and line broadening. In this work we compared the rotational temperature of OH(A), N\(_2\)(C) and N\(_2^+\)(B) and the gas temperature obtained from line broadening of atomic lines of helium (667.82 nm). We compared the obtained gas temperature with gas temperature measurements by Rayleigh scattering for an argon plasma. For argon the rotational temperature of N\(_2\)(C) is overestimating the gas temperature due to a resonance energy transfer between argon metastable atoms and N\(_2\) molecules. The most reliable OES method for the argon plasma is found to be the rotational temperature of OH(A) considering rotational levels up to J = 5.

For the helium and the helium-argon mix, the rotational temperatures of OH(A) and N\(_2^+\)(B) are overestimating the gas temperature significantly. However, in this case, the resonance and van der Waals broadening of the helium (667 nm)-line and the rotational temperature of the N\(_2\)(C) molecule yield reliable gas temperatures.

The Stark broadening of the H\(_\alpha\) and H\(_\beta\) line has been used to obtain electron densities of the plasma jet. However, due to the strong contribution of the van der Waals broadening, the accuracy relies heavily on the accuracy of the gas temperature measurements and gives at best an order of magnitude accurate electron density. For helium a power balance has been used to give a more accurate estimate of the electron density and the discrepancy with line broadening is attributed to induced Stark broadening by the electric field of the plasma.

A molecular beam mass spectrometer (MBMS) system with three pumping stages has been constructed to perform in situ mass spectrometry at atmospheric pressure. The MBMS has been absolutely calibrated for nitric oxide and ozone measurements, while relative densities of ions are obtained.

Changing the plasma power and the duty cycle shows that the nitric oxide and ozone production can be, up to a certain degree, independently changed by varying these two parameters. For a pure argon plasma, without the admixture of molecular gases, densities up to 4 ppm and 6.8 ppm are measured for nitric oxide and ozone, respectively. Furthermore it has been found that the ion density is decaying exponentially with the distance in the far effluent, which shows that, at approximately 10 mm from the nozzle of the plasma jet, the ion density is at least 2 orders of magnitude smaller than the neutral density (of NO and O\(_3\)).

The time resolved power dissipation as well as the spatially resolved gas temperature have been measured in order to investigate what effect a (conductive) substrate has on the RF plasma jet. We found that for substrates with a high conductivity, the power and gas temperature are increasing, when the plasma is in direct contact with the substrate. As long as there is no direct connection between the plasma and the substrate, power and gas temperature do not change. The reason for the change in power is due to the change in impedance, when the sub-
strate is introduced. This leads to a more favourable matching of the RF power, and to more power dissipated in the plasma. This effect is more pronounced for the linear-field configuration compared to the cross-field configuration.

Images captured with an ICCD camera have shown that the time resolved power indicates (by a sudden increase of power) the time point when the plasma is in direct contact with the substrate. The above results indicate that our plasma jet in touching mode increases its plasma dissipated power, its gas temperature and with that most probably the densities of reactive and charged species. Because of this, the RF plasma jet has not been used for the application in touching mode, and has been used with the cross-field configuration to have the least effect of a substrate on the plasma. This allows us to more accurately link the bio-results with the measurements of the plasma parameters.

**Plasma properties of the mono-polar ns pulsed jet**

The similarities between argon and helium plasma bullets operated in ambient air and ambient argon and helium have been investigated. We have shown for the first time that guided argon streamers can be created also in ambient argon. However, the guided streamers only exist in a small parameter window. The reason for the guidance of the streamers is due to the remaining charged and metastable species from the previous pulse, which are creating a channel and cause the guidance. We showed that the diffusion time scale is smaller than the period of the voltage pulse, leaving the majority of the charged and metastable species inside the gas channel. Furthermore, when using helium with admixtures of oxygen as carrier gas in ambient helium, branching is observed. This branching is stable in space and time.

The time and spatially resolved electron density and mean electron energy of a (guided) argon streamer have been measured for the first time using Thomson scattering. For the investigated conditions, a maximum electron density of $7 \cdot 10^{19} \text{ m}^{-3}$ has been measured. The electron density decays after 15 ns to its 1/e-value. A peak mean electron energy of 4 eV has been found, decaying at the same time scale as the electron density for the first 50 ns. The observed fast decay is attributed to the fast drift of the electrons in the high electric field present in the streamer. A significant difference between the emission profile and the much broader electron density profile is observed. The latter corresponds to the pre-ionisation density profile of the previous discharge.

**Plasma interaction with prokaryotic and eukaryotic cells**

The two types of plasma jets (RF and ns-pulsed) have been used for biomedical experiments, to investigate the mechanism of bacteria inactivation and plasma interaction with mammalian cells. To perform the biological measurements, the
time modulated argon RF plasma jet with the cross-field configuration in a non-touching condition is used. As the cells are suspended in the liquid, the main plasma related effect is through plasma induced liquid chemistry.

We have developed a simple 0D solution kinetics model using 30 reactions and the gas phase flux of NO, O$_3$ and OH as input parameters. By comparing the measurements and the modelled concentrations of created species in the liquid we have shown that for the used treatment conditions, reactive nitrogen species, such as HNO$_2$ and ONOO$^-$, are present at concentrations in the liquid phase at which bacteria inactivation has been reported. H$_2$O$_2$ is present at concentrations at which bacteria growth inhibition has been reported. The results of additional measurements suggest synergistic effects between hydrogen peroxide and nitrous acid.

We have successfully found treatment conditions for which bacteria (*Pseudomonas aeruginosa*) were efficiently inactivated while eukaryotic cells (keratinocytes and fibroblasts) showed no significant change in cell activity. It has to be noted that prokaryotic and eukaryotic cells have been treated in the same medium, i.e. saline, while in many cases reported in literature different media are used for prokaryotic and eukaryotic cells. This makes comparison difficult as the plasma induced liquid chemistry depends strongly on the composition of the solution.

The reason for the higher survivability of the eukaryotic cells is due to repair mechanisms against RONS induced cell(-wall) damage. However for longer treatment times, cell viability decreases. In order to investigate the potential of the plasma jet on enhanced cell proliferation, a guided helium streamer jet has been used to treat mouse cells (CMT-cell line). The plasma can be in direct contact, without changing the plasma parameters drastically as in the case for the RF plasma jet. In addition the ability of guided streamers to propagate through tubes, such as endoscopes, could be beneficial for future applications. In this case again a parameter window was found where bacteria are inactivated but cell activity is not significantly reduced.

We have measured several liquid and gas phase parameters with the above established diagnostic methods, showing that with the guided streamer and the used treatment conditions the pH does not drop as much as with treatments of the RF plasma. The amount of ions at the treatment position and part of the acidity is not induced by HNO$_x$ as in the case with the RF jet. Although it seems that due to the similar H$_2$O$_2$ and NO$_x$ concentrations, bacteria inactivation is likely to be caused by similar liquid phase chemistry. An extensive set of measurements has been obtained in order to investigate if cell proliferation is stimulated using the plasma jet (as reported in literature for different plasma and treatment conditions). However no statistically significant effect could be observed for the used treatment conditions.
Summary and outlook

In conclusion, a plasma source has been developed which was operational in a variety of modes. The jet has been characterised by a large set of diagnostics which makes it one of the best characterized systems in the plasma-bio community. Combined with a simple liquid chemistry model and further analysis of reactive species in the liquid, the key parameters of plasma induced bacteria inactivation have been quantified. Furthermore by using the same configuration, for the first time the spatially and temporally resolved electron density and mean energy of streamer discharges have been obtained experimentally. No statistically significant effect on cell proliferation has been observed for the used treatment conditions.

It is the author’s hope that the results presented in this work contribute to the characterisation of the large amount of different plasma jets used in many research groups around the world. A better characterisation will enable to directly compare different plasma jets and the power measurement developed in this work can contribute to this necessary exercise. In addition, it is our hope that the spatially resolved electron density and temperature measurements of the guided streamers are useful to modellers to validate and compare their models with experimental results. Both the characterisation and models are key aspects in the development of a safe and effective plasma source which might become in the future a standard tool in every hospital to heal wounds and help millions of people.
Acknowledgements

Thank you.
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2010–2013, Ph.D.
Eindhoven University of Technology, Department of Applied Physics, group Elementary Processes in Gas discharges. Subject: Atmospheric Pressure Plasma Jets: Characterisation and Interaction with Human Cells and Bacteria, supervised by prof. dr. ir. lic. P. J. Bruggeman and prof. dr. ir. G. M. W. Kroesen.

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