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DEVELOPMENT OF PAPER-BASED MICROFLUIDIC STRIPS FOR QUANTIFICATION OF AMMONIA

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by

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PREFACE

This work was conducted in the Department of Chemistry, Durban University of Technology, Steve Biko Campus, Durban, South Africa from February 2017 to October 2018. The work was performed under the esteem supervision of Dr P.S Mdluli with the assistance of Dr L Madikizela.

DECLARATION

I, **Nonhlazeko Loveday Nxumalo** hereby proclaim that:

This dissertation is entirely my own work, and that all of the references to the best of my understanding, are unequivocally itemized.

This work has not been submitted for a degree at any other university.

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DEDICATION

I dedicate this work to my two girls, Naledi and Yandiswa, your love and support inspired in me the pursuit of excellence and the desire to learn more.

ABSTRACT

Water is one of the most valuable and crucial of life and therefore accurate monitoring and assessment of water resources for sustainability is imperative. Conventional water investigation includes manual gathering of tests, their transportation and resulting examination in the research center. This is time and labour-intensive, costly and requires exceptionally qualified personnel. Sovereign of this procedure empowers more continuous examination, sparing time and cash for analysts, ventures and administering bodies. Consequently, there is requirement for advancement of minimal effort ecological microfluidic paper-based expository gadget that is fundamental for compelling administration of our profitable water assets. This will address the huge and growing demand for low-cost ammonia sensors as legislation becomes more stringent and as more frequent monitoring becomes essential for legislative compliance.

Subsequently, this thesis reports on the development of a low-cost, colorimetric, wax-printed microfluidic paper-based analytical device (µPAD) to detect ammonia in industrial wastewaters. Microfluidic innovation was utilized to facilitate the examination of analytes on the colorimetric explanatory techniques onto a convenient detecting gadget. This therefore empowers the blending of little volumes of analytes with synthetic reagents to form a coloured/hued product in the sight of the analyte of interest. The µPAD fabricated was an oval shaped pattern which was designed on Corel draw software. The hydrophilic segments were made by printing a chromatographic paper with hydrophobic paper sizing agents utilizing a standard Xerox wax printer (Xerox colorqube 8570).

The quantification of ammonia in wastewater was performed on the µPADs using two typical colorimetric methods namely, Nessler reagent and Salicylate. The reaction of ammonia with the Nessler reagent resulted in a brown or intense yellow colour whereas with the salicylate method, the final colour was green. For both methods, the colour intensity increased proportionally with the analyte concentration, and all images of the µPADs were captured and colorimetrically analyzed with ImageJ software for quantification. The analytical performances of the µPAD were linear from 0 to 5 mg L^{-1} with a limit of detection of 3.37 mg L^{-1} and 3.20 mg L^{-1} for the Nessler reagent and salicylate methods respectively. The validity and accuracy of aforementioned methods was supported by the standard UV Visible spectrophotometric method and applied to the measurement of wastewater effluent samples. Wastewater samples were analyzed and the results obtained were similar to those obtained with a spectrophotometric method, demonstrating that the µPAD is suitable to determine ammonia in wastewater.

ACRONYMS

NH_3	Ammonia
NH_4^+	Ammonium ion
K_2HgI_4	Potassium tetraiodomercurate(II)
KOH	Potassium hydroxide
NaOH	Sodium hydroxide
OCl^-	Hypochlorite ion
RGB	Red Green Blue
DWA	Department of Water Affairs
EPA	Environmental Protection Agency
ETP	Effluent Treatment Plant
LOD	Limit of detection
µPAD	Microfluidic paper-based analytical device
SEM	Scanning Electron Microscope
SBP	Southgate Business Park
UIC	UMbongintwini Industrial complex
WWTP	Wastewater Treatment Plant
WHO	World Health Organization

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CHAPTER ONE

OVERVIEW OF THE STUDY

1.1 Background and Context of the study

Environmental protection is a significant matter that occupies a large area of global concern. Many issues are related to environmental protection, such as energy conservation, ecosystem reserve, water purification and wastewater treatment (Hutton and LaRocca 1975). Consequently, the detrimental effects of ammonia waste in waters have resulted in the regulation of the amount of ammonia (NH_3) in wastewater effluent (Tchobanoglous, Burton and Stensel 1991). The South African guideline for ammonia in effluent was 1.5 mg L^{-1} at pH > 8.5 (Nordin *et al.* 2009). Due to the toxicity to fisheries and aquatic life, the European Union has set a safe limit of 0.005 – 0.025 mg L^{-1} ammonia-nitrogen (Chapman and Organization 1996). The determination of ammonia has been the most significant part of water analysis. As a result, all types of water supplies, effluents and sewage have to be tested frequently for occurrence of ammonia (Kruse and Mellon 1953).

In particular, human industrial activities, including the application of NH_3 for cleaning products and fertilizer as well as biological waste treatment that produces NH_3, have introduced high concentrations of ammonia into the environment. The main concern is that NH_3 can bring serious environmental problems, such as eutrophication and dissolved oxygen depletion in the receiving water body, leading to the destruction of the living environment of aquatic organisms (Jorgensen 1975; Colt and Huguenin 2002; Gerber, Opio and Steinfeld 2007). So, there is doubt that potential occurrence of ammonia in the environment has increased the need for wastewater management and treatment, driving the demand for wastewater treatment to higher levels. Essentially important, the quantification and detection of ammonia from industrial wastewater prior to its discharge into natural water system is necessary in order to preserve proper water quality for normal aquatic life.

Numerous methods for the determination of ammonia have been developed for samples with complex matrices, such as sample distillation with subsequent colorimetric, potentiometric or titrimetric measurement being the most frequently used (Thomas *et al.* 1997; Molins-Legua *et al.* 2006). Most of these methods are either laborious, expensive or include reagent manipulation steps and require the highly trained technician. The development of inexpensive methods for routine measurement of the ammonia concentration in domestic, agricultural and industrial effluents, which are the main sources of ammonia contamination of the hydrosphere, is of great importance for ammonia pollution control of environmental waters. The developed method should be simple, high sensitive, optical ammonia sensor that is operable at ambient condition, capable of detecting ammonia in parts per million (ppm) levels (Srivastava, Verma and Jain 2012).

Furthermore, Meredith and co-workers (2016) stressed that there was a need for low-cost technologies that could detect the concentration of ammonia in the environment. Such technologies should provide scientific data quickly, be easily applied in the field and thus they could provide timely data regarding the extent and magnitude of pollution (Molins-Legua *et al.* 2006; Meredith *et al.* 2016). It has been suggested that a low-cost microfluidic paper based analytical device (μPAD) offers an opportunity to tackle this need (Martinez *et al.* 2010; Jayawardane, McKelvie and Kolev 2015). Paper-based devices are of low cost, disposable, portable, easy to store because of their small size, and provide high optical contrast for colorimetric detection (Bruzewicz, Reches and Whitesides 2008; Tomazelli Coltro *et al.* 2014). The processing of μPADs data can be done remotely because the colorimetric testing results could be scanned or photographed and transmitted electronically, which is particularly beneficial in environmental monitoring and analysis in remote areas by unskilled staff (Martinez *et al.* 2008). This study describes the development of a low cost μPAD for accurate and reproducible quantification of ammonia in wastewaters based on colorimetric method.

1.2 Problem Statement

Globally, because of rapid industrialization and increasing population density, industrial wastewaters are large sources of effluents that are discharge into receiving water bodies daily. Ammonia is widely used in the production of fertilizers, plastics and explosives, resulting in large quantities of wastewater containing ammonia. It is therefore one of the most hazardous water pollutants discharged into water receptors through industrial effluents.

Consequently, when discharged to receiving bodies of water, ammonia causes algal blooms that exhaust dissolved oxygen and release toxins, destroying aquatic life. Thus, controlling ammonia discharges from wastewater treatment can make significant contribution to reducing it environmental impact and is subject to a growing number of directives. To therefore safeguard public health and prevent environmental impact, regulatory agencies are requiring industry to treat ammonia in their effluents to meet rigorous requirements.

1.3 Aim of the study and research objectives

The aim of this project is to develop an inexpensive, disposable and highly selective µPAD for the quantification of the ammonia contents in the industrial wastewater effluent, thereby ensuring that it complies with environmental standards of South Africa.

Objectives are to:

- fabricate colorimetric chromatographic paper (µPAD).
- optimise the µPADs using ammonia standards.
- quantify ammonia in wastewater using the optimised µPAD.
- validate the µPAD against the existing methods such as titration.
- validate colorimetric sensor against spectrophotometer.

1.4 Research hypothesis

Null hypothesis, H_0: The new microfluidic paper-based device is a suitable alternative quantification device for detecting trace levels of ammonia.

Alternate hypothesis, H_1: The new developed microfluidic paper-based device is not a suitable alternative device for detecting trace levels of ammonia.

1.5 Rationale/Significance of the study

Meredith and co-workers (2016) pointed out that analytical chemistry still heavily relies on titration and expensive equipment- which increases the cost of analysis overall (Meredith *et al.* 2016). From an economic and geographical perspective, there is a need to explore the use of µPADs as a low-cost innovative technology in analytical chemistry, particularly for rapid and easy analysis of heavy and hazardous chemicals such as ammonia.

1.6 Assumptions

The following assumptions were made in relation to this study:

- All data collection instruments are valid and reliable based upon their frequent use and calibration period at the Durban University of Technology.

- The new microfluidic paper-based device is user friendly and portable than the currently used methods; hence it will be a more viable option for field testing of ammonia.

1.7 Delimitation/Scope of the study

The study was restricted to effluent treatment plant (ETP) dam situated inside the uMbogintwini Industrial Complex (UIC) and Southgate Business Park, which is situated in south of Durban city in KwaZulu-Natal Province, South Africa.

Wastewater flowing only from the industrial activities performed within UIC was considered. Samples of wastewater were collected from the ETP dam in order to detect and quantify the concentration of ammonia using microfluidic paper-based analytical devices (µPADs).

1.8 Study site

The study was directed inside the effluent treatment plant dam sited at UIC and the contiguous Southgate Business Park (SBP). The UIC which is expected to experience a name change to Acacia Operational Services is a 350 hectare industrial, and is home to many large recognized industries like Huntsman Tioxide, Evonik, Dulux, SA Bioproducts (Anchor Yeast), KK Animal Nutrition, and many subsidiaries of AECI. The UIC is situated at the south of Durban, along the National Highway (N2) and is five minutes’ drive south of the old Durban International Airport.

The proprietors (Acacia) oversee and supply mass utilities, for example, power, steam, gas, water, gushing treatment, fire insurance, security, and give other particular administrations. Acacia operation services are the possessor of the complex and are the only industry licensed to discard wastewater from UIC to the Kingsway Sea. Thus, all other industries within UIC are obligated to discharge their wastewater through the channel of the Acacia effluent treatment plant.

1.9 Alignment of National Strategies

Literature documented that South Africa has a shortage of skills workforce which could negatively impact on the economy growth. More so, and as revealed by the government report, more than 50% of the shortage of skills are in engineering and science, particularly chemistry. Part of Department of Higher and Training Education (DHET) efforts is to improve scientific literacy and engaged in research and innovation, which will help bridge the gap in the shortage of skilled workforce in South Africa. As a consequence, the DHET together with the Department of Science and Technology (DST) have identified core priority area to achieve the said mandate. Chemistry has been listed among these scarce disciplines to help in sustaining economic growth as well as improving the overall academic outlook of the nation. The researcher envisaged that the development of a simple innovative microfluidic device in the quantification of ammonia will help in achieving the goal of DST in terms of positioning South Africa as a global innovative country, while at the same time, achieving an improvement in the shortage of scarce skills.

1.10 Structure of the Dissertation

This thesis is divided into five chapters as follows:

Chapter 1: Overview of the study

Chapter one provides concise foundation of the study; distinguishes the exploration issue and expresses the point and targets of the study. The speculations, suspicions, delimitation and extent of the study is sketched out. The structures of the thesis are quickly clarified.

Chapter 2: Literature review

In this chapter an overview of the literature based on the development of microfluidic paper is covered. The review covered, the overview of ammonia in wastewater and it health effects. The discussion on the characteristics of ammonia and applications was also considered.

Chapter 3: Research methodology and design

This chapter centers on the research methodology utilized in this study. It incorporates a discourse of the research design, the research instrument, the exploration instrument, information accumulation strategies, information investigation systems, and the techniques used to guarantee that the exploration is substantial and solid.

Chapter 4: Presentation, analysis and discussion of results

The consequences of the study are contextualized inside the writing explored, and where fitting, are contrasted and the aftereffects of comparative examinations led somewhere else. The fundamental examination of the information is introduced graphically by means of visual diagrams and pie outlines, and tables are likewise exhibited to help in the investigation and discussion of the outcomes.

Chapter 5: Review, conclusion and recommendations

This chapter provides the conclusions drawn from the study. It likewise contains an outline of the key discoveries of the study, makes proposals for further research and distinguishes any restriction.

CHAPTER TWO

LITERATURE REVIEW

2.1 Literature review

A literature study using secondary sources of information was conducted with the objective of establishing, assembling and integrating theory with regard to the development of µPADs for the quantification of ammonia in wastewater. In this regard, academic journals, newspaper and magazine reports, as well as government and non-governmental organizations reports and publications were accessed. The literature related to the techniques and methods of the quantification of ammonia in the environment was reviewed. The review introduces ammonia in terms of its physical and chemical characteristics. Subsequently, the health and environmental concerns related to the presence of ammonia in the atmosphere is discussed. Overall, different methods of quantifying ammonia in the laboratory are explained. This section concluded by evaluating the use of a simple microfluidic device as a cost effective method for quantifying ammonia.

2.2 Overview of ammonia and its associated health effect

Ammonia is a colourless, alkaline gas at ambient temperature and pressure, with a distinct pungent odor. Several studies have reported on ammonia in terms of its chemical characteristics (toxic nature, corrosiveness, flammability, colourless, pungent smell) and physical properties (boiling point = -33.4 ^{o}C and pressure = 760 mmHg, density = 61 b gal^{-1}, odor threshold = 17 ppm) (Erisman and Schaap 2004; Timmer, Olthuis and Berg 2005; Sutton *et al.* 2008). The properties of ammonia have significantly contributed to the nutritional needs of terrestrial organisms, as it serves as the precursor for food and fertilizer. As reported by Sutton et al (2008), ammonia is used either directly or indirectly for the synthesis of pharmaceutical products, refrigerant gas, for purification of water supplies, and in the manufacture of plastics, explosives, textiles, pesticides, as well as in many commercial cleaning products (Sutton *et al.* 2008).

Ammonia is one of the most abundant nitrogen-containing compounds in the atmosphere characterised by it irritant and pungent smell (Gerber, Opio and Steinfeld 2007). It presence on the environment is due to natural processes and industrial activity, including certain types of intensive farming (Haber 2002; Erisman *et al.*

2007). It is used in agriculture, explosive and textile industries, and commercial products, including various household cleaning products (Fedoruk, Bronstein and Kerger 2005). Although common in nature and widely used, ammonia is both caustic and hazardous in its concentrated form (Pagans *et al.* 2006). As such, it is classified as an extremely hazardous substance, which is subject to strict reporting requirements by facilities that produce, store, or use it in significant quantities.

Notably, ammonia is emitted from different sources namely, animal manure, traffic and fertilizer application. Its role in acidification and eutrophication as well as its impact on ecosystems and water quality is well documented (Dalton and Brand-Hardy 2003). Hence, as reported in the United States Emergency, and Planning Community Right to Know-Act, ammonia is subject to strict reporting requirements by facilities which produce, store, or use it in significant quantities (Palenchar 2008). Furthermore, studies have shown that exposure to high ammonia concentrations is a serious health threat, in particular, occupational exposures to ammonia have commonly been associated with sinusitis, upper airway irritation, and eye irritation (Morales-Bahnik, Czolk and Ache 1994). For instance, concentration levels near intensive farming can be higher than the allowed exposure limit of 25 mgL^{-1} (Brautbar, Wu and Richter 2003). This could lead in unhealthy situations for farmers and animals inside the stables, where the concentrations are highest. Similarly, and as revealed by (Roney and Llados 2004; Kerger and Fedoruk 2015), acute exposures to high levels of ammonia have also been associated with diseases of the lower airways and interstitial lung. Therefore, it is sensible to accurately quantify the concentration of ammonia due to its potential hazard to human beings, even at small concentrations. As a consequence, Rodriguez et al, advised that real-time environmental monitoring of ammonia is a critical issue in closed environments (Rodríguez *et al.* 2014).

Of concern, Karri et al (2018) documented that the presence of ammonia in wastewater was a major concern for environment conservation and human health (Karri, Sahu and Chimmiri 2018). They reported that ammonia was formed by bacteria decomposition processes of organic matter (Halling-Sørensen and Jorgensen 1993). it is therefore sensible to quantify the amount of ammonia that is present in wastewater effluent in order to ensure that the concentration of ammonia in the effluent meet the stringent requirement of wastewater treatment plants (WWTPs) needed for discharging it to the environment.

2.3 Ammonia Impacts: Environmental and human health impacts

2.3.1 Environmental impact of ammonia

Effects of ammonia gas and ammonium particles on climate and ecosystem are important issues. Ammonia can impact the environment in various ways; these include eutrophication of surface water, soil acidification, fertilization of vegetation and changes in ecosystem.

2.3.1.1 Ecosystem changes

An ecosystem is a natural system involving of plants, animal, and other microorganisms functioning together in a balanced relationship. One of the major air pollutants is ammonia which is increasingly being recognized because of its role in regional-scale tropospheric chemistry and its effects when deposited into ecosystems (Park *et al.* 2006). The changes in ecosystems because of ammonia deposition occur through a combination of all the above-mentioned processes. When changes in ecosystems occur, the natural balance of a system is disrupted and fragile plant and animal species can be replaced by non-native or N-responsive species (Constable *et al.* 2003). The disruption of an ecosystem can cause it to adapt by changing (positive or negative outcome), or a disruption may lead to the extinction of the ecosystem (Erguder *et al.* 2009). Therefore, ammonia should be properly removed from wastewater streams to protect environment and save its components (Sutton *et al.* 1998).

2.3.1.2 Eutrophication

Eutrophication due to high levels of ammonia and nitrogen in wastewater is one of the main environmental problems associated with the improper disposal of wastewater (Jafarpour *et al.* 2010). In aquatic environments, enhanced growth of choking aquatic vegetation or algal blooms disrupt normal functioning of the ecosystem, causing problems such as a lack of oxygen in water, needed for fish and other aquatic life to survive (Klimont 2001; Ansari, Gill and Khan 2010; Sheppard *et al.* 2011).

2.3.1.3 Acidification of soil

At hazardous waste sites, ammonia can be found attached to soil particles. As it reaches the soil surface, it generally reacts with water in the soil and is converted into its ionic form, ammonium (NH_4^+), and absorbed to the soil. The ammonium in the soil eventually disassociates or is nitrified into nitrite (NO_2^-) or nitrate (NO_3^-) by nitrifying bacteria, releasing hydrogen ions (H^+) into the soil. The excess H^+ ions eventually lead to the formation of an acidic soil environment, if not taken up by biomass and converted to methane. The nitrogen left over in the soil will either be taken up by plants, stored in the soil, returned to the atmosphere, or will be removed from the soil in runoff or leaching (Bouman *et al.* 1995; Ritz, Fairchild and Lacy 2004; Duong 2016).

2.3.2 Human health impacts

Ammonia affects human and animal health both as a gas and as a particulate. The particulate form of ammonia has broader implications for the general public, whereas the gaseous form is a localized concern for the health of animals and agricultural workers. The immediate health effects of ammonia exposure can be summarized as follows:

2.3.2.1 Ingestion

Being exposed to high concentration of ammonia solution can usually lead to corrosive damage on the mouth, throat and stomach (Dobler, Gibbons and Evtodienko 2006). Ingestion of ammonia does not normally result in systemic poisoning (Roney and Llados 2004).

2.3.2.2 Skin or eye contact

Ammonia is a corrosive substance and the main toxic effects are restricted to the sites of direct contact with ammonia (i.e., skin, eyes, respiratory tract, mouth, and digestive tract). Exposure to low concentrations of ammonia whether in air or solution may produce rapid skin or eye irritation. Higher concentrations of ammonia may cause severe injury and burns (Timmer, Olthuis and Berg 2005). Contact with concentrated ammonia solutions such as industrial cleaners may cause corrosive injury including skin burns, permanent eye damage or blindness (Welch 2006). The full extent of eye injury may not be apparent for up to a week after the exposure. Contact with liquefied ammonia can also cause frostbite injury (George *et al.* 2000). Ammonia has not been classified for carcinogenic effects by EPA, Department of Health and Human Services (DHHS) (NTP), or the International Agency for Research on Cancer (IARC) (Close, Catlin and Cohn 1980; Swotinsky and Chase 1990).

2.3.2.3 Inhalation

Gaseous ammonia is a highly hydrophilic base that has irritant properties when inhaled. It is therefore classified as corrosive, toxic and dangerous for the environment and humans as well (Swotinsky and Chase 1990). Exposure to high concentrations of ammonia in air causes immediate burning of the nose, throat and respiratory tract (Sundblad *et al.* 2004). Accidental exposures to high concentrations of ammonia gas have resulted in nasopharyngeal and tracheal burns, airway obstruction and respiratory distress, and bronchiolar and alveolar edema (Brautbar, Wu and Richter 2003). Inhalation of lower concentrations can cause coughing, and nose and throat irritation (Ryer-Powder 1991). The odor of ammonia provides adequate early warning of its presence, but ammonia also causes olfactory fatigue or adaptation, reducing awareness of one's prolonged exposure at low concentrations (De Bode 1991). Children exposed to the same concentrations of ammonia vapor as adults may receive a larger dose because they have greater lung surface area-to-body weight ratios and increased minute volumes-to-weight ratios. More appealing, they may be exposed to higher concentrations than adults in the same location because of their shorter height and the higher concentrations of ammonia vapor initially found near the ground (Swotinsky and Chase 1990).

2.4 Standard limits of ammonia in wastewater

Many countries and organizations have enforced stringent standards in recent years for wastewater containing ammonia discharge due to the concern of ammonia pollution. In natural surface and ground water, ammonia can be found at concentrations as low as 10 µg L^{-1} (Association *et al.* 1915; Brooke and Thursby 2005). As stated by the World Health Organization (WHO), the acceptable limit of ammonia normally found in wastewater effluent is 1.5 mg L^{-1} for potential wastewater stream (Chapman and Organization 1996; Patterson 2001; Organization *et al.* 2009; Qin, Gao and Chen 2012). The unionized ammonia limit is 0.2 mg L^{-1} (Zadorojny, Saxton and Finger 1973; Bouwer and Idelovitch 1987). Accordingly, the associations concerned about drinking water quality and numerous administrations have given careful consideration for ammonia restriction in the drinking water. Associations that limit alkali focus in drinking water are in the range 0.3-1.5 mg L^{-1}, which demonstrates that ammonia involves worry that ought to be dealt with well (Karri, Sahu and Chimmiri 2018). In order to achieve these limits, wastewater treatment plants ought to be enhanced with the end goal to change over nitrogen mixes to less harmful form (Organization *et al.* 2009). Moreover, in view of the health effects of ammonia, federal and provincial governments of South Africa have issued numerous guidelines to synchronize the concentration of ammonia in dischargeable industrial wastewater and drinking water (Hart and Allanson 1984; Roney and Llados 2004). Current South Africa water quality guidelines state that ammonia in wastewater treatment plant effluent should not exceed 0.025 mg of ammonia/kg as high concentration may affect blood pH, osmo-regulation, increase urine flow and/or may adversely affect the oxygen-binding capacity of hemoglobin (Adler *et al.* 2007; Hemson 2016).

2.5 Wastewater effluents

As previously stated, ammonia is one of the most dominant pollutants in the aquatic environment because of its relatively highly toxic nature and its presence in surface water systems. It is discharged in large quantities in industrial, municipal and agricultural wastewaters (Tilche and Orhon 2002; Park *et al.* 2006; Karri, Sahu and Chimmiri 2018). Consequently, measuring the toxicity of aqueous ammonia is challenging due to the differing nature of the two common forms (gaseous or liquid state) of ammonia when dissolved in water (Hutton and LaRocca 1975). The relative concentrations of ionized and unionized ammonia in an ammonia solution are principally a function of pH, temperature and ionic strength of the aqueous solution

(Roney and Llados 2004; Pagans *et al.* 2006). However, the concentration of ammonia can vary at any given moment depending on the water conditions. The presence of ammonia from industrial wastewater may reduce the yield of crops and the growth of plants and it can be harmful to the aquatic living organism as well (Lefebvre and Moletta 2006). Thus, it can bring about serious environmental problems, such as eutrophication and dissolved oxygen depletion in the receiving water body, leading to the destruction of the living environment of aquatic organisms (Sutton *et al.* 2008; Karri, Sahu and Chimmiri 2018). This can be a challenge for regulators, as well as companies whose manufacturing operations result in wastewater with measureable ammonia levels. This is resolved by measuring the total ammonia content of the water. In particular, wastewater can become toxic depending upon the time of day and the season. Companies must be vigilant to prevent discharging excessive amounts of NH_3 in their wastewater to avoid penalties. Essentially important, the determination of ammonia from industrial wastewater prior to its discharge into natural water system is necessary in order to preserve proper water quality for normal aquatic life. Thus, controlling ammonia discharges from wastewater treatment can make a significant contribution in reducing its environmental impact and is subject to a growing number of directives.

According to Templeton and Butler, wastewater primarily consists of pure water (above 95%), with impurities below 5% (Templeton and Butler 2011). Various processes can be implemented for treatment depending on the nature and extent of contamination (Sonune and Ghate 2004). The treatment of wastewater is not only essential for our own health but also to keep our environment clean and healthy. The objective of treatment is to produce a disposable effluent without causing anguish to the surrounding environment and, to avoid pollution (Khopkar 2007). In the absence of proper wastewater treatment, many ecosystems would be critically damaged once the inadequately treated water gets recharged back into the environment (Thomas *et al.* 1997; Qin, Gao and Chen 2012). Consequently, untreated wastewater generally contains many pathogenic or disease-causing, microorganisms that bide in the human intestinal gut or that may be present in certain industrial waste (Tchobanoglous, Burton and Stensel 1991).

Treatment of wastewater is essential in order to improve and purify the water, removing some or all of the contaminants, making it fit for reuse or discharge back to the environment (Akpor 2011). Consequently, after the treated wastewater may be discharge to surface water, such as rivers or the ocean, or to groundwater that lies beneath the land surface of the earth (Bouwer 2002). Proper treatment of wastewater assures that acceptable overall water quality is maintained. In many parts of the world, health problems and diseases are often caused by discharging untreated or inadequately treated wastewater (Rajaram and Das 2008). Such discharges result in the spreading of disease, fish kills, and destruction of other forms of aquatic life (Qadir *et al.* 2010).

2.5.1 Industrial wastewater

Industrial wastewater refers to the industrial production process of wastewater discharge (Jorgensen 1975; Ho *et al.* 2012). This type is discharged by manufacturing processes, industrial plants and commercial enterprises. The character of industrial wastewater depends on the type of industry using the water. Process wastewater can contain rinse waters including such things as residual acids, plating metals, and toxic chemicals. Industrial wastewater characteristics vary according to the industry type, but, in general, ammonia concentration is higher than in the domestic wastewater (Jorgensen 2002; Rajkumar and Palanivelu 2004).

According to Mema, water pollution due to discharge of untreated industrial effluents into water bodies is a major problem in the global context (Mema 2010). The problem of water pollution is being experienced by both developing and developed countries (Ujang and Henze 2006). Particularly, the character of industrial wastewater depends on the type of industry using the water. Water used during industrial processing tends to accumulate various contaminants which are released at a particular stage during the industrial process. Along with the increase in industrial activity there is an increase or occurrence of pollution in the nearby environment through industrial effluents and gaseous emissions (Tilche and Orhon 2002; Patwardhan 2017). Some industrial wastewaters can be treated the same as domestic wastes without difficulty. Others may contain toxic substances or high percentages of organic materials or solids which make treatment difficult. In such cases, the industrial plant may have to pre-treat its wastewater to remove these pollutants or reduce them to treatable levels before they are accepted into a publicly-owned treatment facility (Rowe and Abdel-Magid 1995; Phiri *et al.* 2005). Hence, the monitoring of wastewater quality is a subject of great importance around the world as better understanding of treated and untreated effluent will lead to better treatment plant control measures (Bourgeois, Burgess and Stuetz 2001).

According to Myers 2017, the presence of ammonia in wastewater in recent years has resulted in a critical apprehension in numerous countries (Myers 2007; Karri, Sahu and Chimmiri 2018). Therefore, wastewater quality intended to be discharge to the environment must be ensured for ammonia contents in the final wastewater effluent, thereby complies with environmental standards of South Africa (Pearce and Ollermann 1998).

2.5.2 Water pollution due to Industrial activities

The contamination of water is due to presence of various pollutants in it, which becomes dangerous for the living beings and thus, causes several diseases and harmful effects when consumed in different ways (Mathuthu, Mwanga and Simoro 1997; Templeton and Butler 2011). Consequently, due to the presence of high amount of toxic, carcinogen, and teratogen of metals, researchers are highly concerned with its effect on the environment and health of mankind (Singh and Kalamdhad 2011).

Various industrial activities are a major contributor in polluting the ground and river water resources (Patwardhan 2017). The character of industrial wastewater depends on the type of industry using the water. Industries use water for diverse purposes, such as for manufacturing of goods, heating, cooling, as a carrier of raw material, as a carrier solvent of waste, for fire fighting, to lawn sprinkling and gardening, and for use in canteens and toilets (Rajaram and Das 2008). However, only a small fragment of the supplied water is present in the end product or is lost by evaporation, the rest is converted into industrial wastewater (Ho *et al.* 2012). The Industrial discharge in various form such as water mixed with harmful chemicals, small metal chips, plastic particles, paints, hot water etc. get mixed with the ground water or running river water, it contaminates the large amount of water table and make it unsuitable for normal use (Sonune and Ghate 2004; Ho *et al.* 2012). Contaminated and polluted water give rise to various health hazards and sometimes even results into life threatening diseases (Rajkumar and Palanivelu 2004; Luo *et al.* 2015). Therefore, disposal of treated wastewater is a dominant step that an industry has to follow in order to ensure that the exquisite ecological balance of the environment is not disturbed.

Wastewater reacts as an agent for polluting the air and environment through diverse means. Polluted water sources cause abundant spread of water pollution. As per the environmental policies, all industries should discharges their harmful wastewater only after proper treatment, however, due to inadequate implementation many industries release their waste water without any treatment resulting into water pollution (Sonune and Ghate 2004; Qin, Gao and Chen 2012).

2.5.3 General characteristics of effluents and water quality

The term water quality describes the physical, chemical and biological characteristics of water (Diersing and Nancy 2009) and it fitness for use and ascertaining it quality is crucial before use for various intended purposes such as potable water, agricultural, recreational and industrial water uses (Sargaonkar and Deshpande 2003). The quality of water is affected by human activities which include agricultural activities, urban and industrial development, mining and recreation as well as by natural processes such as seasonal variation, climatic changes and the types of soils, rocks and surfaces through which it flows.

Water is one of the most valuable and crucial resource of life. Therefore accurate monitoring and assessment of water resources is compulsory for sustained water resource management (Hodgson and Manus 2006). Monitoring the quality of our waters influences many areas of society including public health and the general economy due to the industrial and agricultural processes it affects. The assessment of water quality is censorious for pollution control and the protection of surface and ground waters (Novotny 1994). The regimen of monitoring and assessment is enabled by comparison with established water quality parameters which are broadly categorized as physical, chemical and biological parameters.

2.5.4 The impact of industrial release to seawater

Industrial discharge contaminants flow through river, or directly discharged to the sea. Some are gather, interact and settle with the living organism, plant and sediment and finally reach the coastal and ocean. Plants and living organism in the ocean are important food sources for human intake. Contaminants may then enter human food chain and accumulate in fishes, molluscs (octopus, shellfish, and cockle), crustaceans (shrimp, crab, and lobster), seaweed, sea cucumber and etc. Therefore, it is essential to understand the effect to aquatic environment.

2.6 Analytical techniques and methods for analysis of ammonia

Different approaches for NH_3 determination can be found elsewhere (Molins-Legua *et al.* 2006; Galvão *et al.* 2013). In particular, one can find a great variety of methodologies based on flow injection analysis (FIA) and related techniques coupled to potentiometric (Ferreira, Lima and Rangel 1996), fluorimetric (Mana and Spohn 2000; Duong and Rhee 2014), colorimetric (Amirjani and Fatmehsari 2018) and spectrophotometric (Almeida *et al.* 2011; Liang *et al.* 2016) detection. Colorimetric method is frequently used method of detection. The most recent developments on NH_3 determination also include the use of lab-in-syringe Head-Space Single-Drop Micro-extraction (Šrámková *et al.* 2016), microfluidic paper-based analytical devices (Tomazelli Coltro *et al.* 2014; Jayawardane, McKelvie and Kolev 2015) and microchips (Zhu *et al.* 2015).

However, the aforementioned techniques are complicated, time consuming, highly expensive and required technical expertise. Furthermore, Sutton and co-workers (2008) alleged that these techniques are liable to pre-analytical errors due to sample transportation, storage, and handling (Sutton *et al.* 2008). Consequently, from an economic and geographical perspective, there is a need to explore the use of μPADs as a low-cost innovative technology in analytical chemistry, particularly for rapid and easy analysis of heavy and hazardous chemicals such as ammonia in any part of

South Africa without necessary needing a laboratory to actualise the experiment. Srivastava, Verma and Jain (2012) moots that the µPADs is a portable, high sensitive and optical ammonia sensor operable at ambient condition and capable of detecting ammonia in parts per million (ppm) levels (Srivastava, Verma and Jain 2012). Accordingly, the development of a low-cost, simple method for the monitoring and detection of ammonia concentration is therefore of great importance in order to control the pollution of ammonia on the environment (Jayawardane, McKelvie and Kolev 2015). The µPADs provide high optical contrast for colorimetric detection. Colorimetric detection has been the most widely employed technique for paper-based analytical devices due to the advantages of visual readout, straightforward operation and superior stability (Amirjani and Fatmehsari 2018). Colorimetry is perhaps the most popular since only a scanner or digital camera and software for image processing are necessary for quantitative analysis (i.e. the device and the detection system are small, inexpensive and easy to use).

2.7 Microfluidic paper-based analytical devices

Microfluidic device has been used in analytical and clinical chemistry for centuries. Nowadays there is a growing trend to use it as substrate for sensors due to its several inherent advantages including; a small footprint, small reagent volumes generating small waste volumes, and increased portability and accessibility for detection relative to traditional laboratory testing methods (Sackmann, Fulton and Beebe 2014). µPAD have become analogous with common paper tests like the pH test strip, HIV test kits and home base pregnancy test, as they operate on a similar principle and are an example of point of care diagnostics (Hu *et al.* 2014). While microfluidic technology has continued to develop in an academic research setting, a considerable gap has developed between their use in the lab and the ability to deploy devices in real world settings (Carvalhal, Carrilho and Kubota 2010; Nie *et al.* 2012; Caicedo and Brady 2016). However, it has shown promise for point-of-interest testing, which refers to testing at the location where information is required rather than in a laboratory. Martinez et al (2010) mooted that µPAD are simply operated and they do not require any supporting equipment or external power and they have the ability to produce results that are easily interpreted (Martinez *et al.* 2010).

Even though most research on µPADs has been focused on point of care diagnostic devices (Dou *et al.* 2015). There has been a trend towards using µPADs in applications where an abundance of analyte is available (Cate *et al.* 2014; Yang *et al.* 2016), such as environmental analysis, specifically for water quality testing (Meredith *et al.* 2016), as well as food and water analysis (Busa *et al.* 2016). Recently, µPADs have received considerable attention since the world health organization (WHO) suggested that they are promising diagnostic tools for the developing world (Blankenstein 2000; Yager *et al.* 2006; Martinez *et al.* 2010). µPADs offer many advantages as simple and portable platforms that require only a drop of target sample for detecting various analytes such as proteins, environmental contaminants, pathogens, chemicals, heavy metals, and drugs (Martinez *et al.* 2007; Ballerini, Li and Shen 2012). Depending on the main goal to be achieved in paper-based sensors, the fabrication method can be tuned to fulfill the needs of the end-user. The choice of the proper technique has to consider some factors including material costs, fabrication process simplicity and the intended applications of paper-based microfluidics devices (Liana *et al.* 2012).

Consequently, µPAD significantly represent a new generation of lateral-flow chemical assays exploiting hydrophobic barriers patterned onto paper. These barriers direct flow of aqueous solutions on the paper in a desired manner so that specific chemical assays may be conducted rapidly and efficiently (Carrilho, Martinez and Whitesides 2009). Equally important, Martinez et al alleged that paper substrates are easy to use because the flow is generated via capillary action (Martinez *et al.* 2007; Martinez *et al.* 2009). Reagents infused in ‘detection zones’ on the µPAD allow analytes to be quantified by visual assessment using an external optical reader (i.e. camera, scanner) (Shen, Hagen and Papautsky 2012).

The implementation of µPADs is to supply a low-cost, easy to use, and portable analytical platform for assays, either multi-analyte or semi-quantitative or even quantitative, in order to give people living in the developing world affordable disease diagnosis and environmental monitoring tools (Li, Ballerini and Shen 2012).

2.8 Portability and user-friendliness of µPADs

The various aspect of paper microfluidics shows a great potential in analytical and clinical chemistry because of it high abundance, low cost and it natural ability of paper to flow fluids has shown its versatility in various fabrication methods (Martinez *et al.* 2010; Dungchai, Chailapakul and Henry 2011). Following the invention of paper chromatography in the early 20th century, diagnostic devices based on paper are becoming more recognized substrate materials (Comer 1956; Yu *et al.* 2011). Due to its natural, high biocompatibility, biodegradability, disposability and chemical and biochemical inertness is particularly important for the immobilization of reagents. The key points of the µPAD which makes it suitable for use are shown in (**Fig 2.1**) (Jagadeesan, Kumar and Sumana 2012; Yetisen, Akram and Lowe 2013).

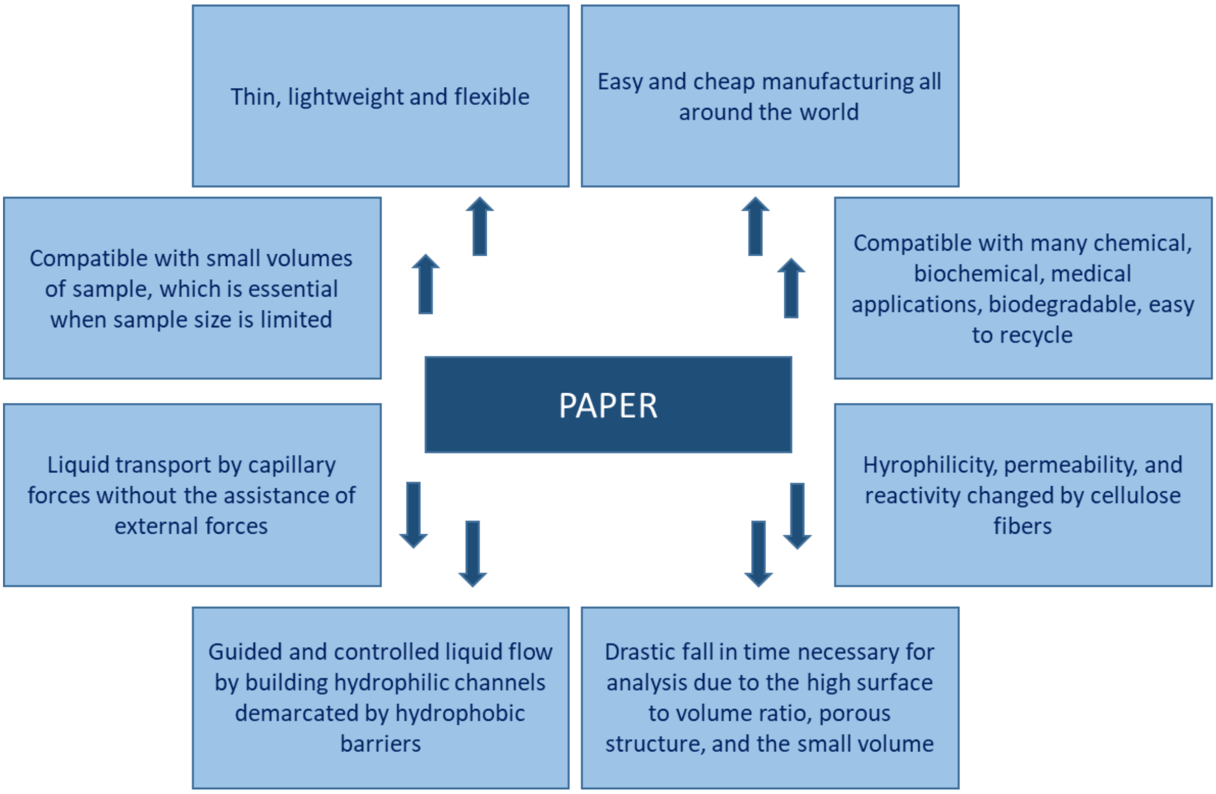


Figure 2.1 *Key purposes of paper for microfluidic paper-based systematic gadget*

Since µPADs are known for their portability to the field, the risk of contamination or degradation of the analyte is significantly reduced. The response time for the analysis is quicker with the use of µPADs. As long as the µPADs are user-friendly and portable, the benefits mentioned above can be applied. Some of the methods that were developed to make μPADs user friendly include using smartphones (Shen, Hagen and Papautsky 2012; Lopez-Ruiz *et al.* 2014), portable cameras and portable scanners as the reading devices (Martinez *et al.* 2008; Jayawardane, McKelvie and Kolev 2015). For example, Sicard and his group (2015) developed a paper based device in conjunction with a smartphone application to provide the organophosphate pesticide concentration in water resources (Sicard *et al.* 2015). Usage of a smartphone can be beneficial not only for image capturing, but also for data collection, storage and sharing-on-line in real time (Sicard *et al.* 2015). Further, a quick response (QR) code containing information about the analyte that enabled the smartphone to read μPADs and output data was developed (Santhiago, Henry and Kubota 2014).

2.9 Paper for microfluidic patterning

Paper has become a budding platform as there is a global demand for simple, disposable and cheap substrates for sensors. Apart from that, it also confers some benefits compared to other type of substrates such as glass (Nery and Kubota 2013). Such advantages include its readily availability and low cost, ease of pattern into discrete hydrophilic and hydrophobic zones using printing or cutting technologies (Dungchai, Chailapakul and Henry 2011). Furthermore it is able to wick fluids by capillary action without external power source, easy to transport because of its lightweight, and disposability (Govindasamy *et al.* 2012; Xia, Si and Li 2016). A paper is made from cellulose, which is an abundant polymer. It therefore presents additional advantages, including biodegradability, biocompatibility, three-dimensional fibrous structure, and mechanical properties which allow capillary flow (Nery and Kubota 2013). Cellulose paper has naturally hydrophilic properties and can transport fluids via the capillary motion without having to apply external pumps or electric power (Dou *et al.* 2015). The thickness, basis weight, porosity, and specific surface area are some of the important physical properties of papers. The hydrophobicity of the paper surface is another important property, particularly for paper-based microfluidics.

Furthermore, a large portion of the burden associated with developing microfluidic devices capable of performing on-site or in-the-field measurements cost effectively lies in the platform material (Cogan *et al.* 2014; Jayawardane, McKelvie and Kolev 2015; Yang *et al.* 2016). Paper as an analytical platform material, has the advantage of providing inexpensive, power-free fluid manipulation, easily modifiable and deployable approach for microfluidic device development (Yetisen, Akram and Lowe 2013; Cate *et al.* 2014). Thus, paper is selected as a first-choice substrate for disposable sensors. In addition, paper may also be modified chemically to incorporate different functional groups that allow for immobilization of proteins, DNA, or small molecules (Credou and Berthelot 2014).

Filter paper has been widely used in developing paper-based biosensor or paper-based microfluidics due to its large porosity and specific surface area (Ballerini, Li and Shen 2012). According to, Liana (2012), Whatman chromatography paper is the most popular among many types of the cellulose papers (Liana *et al.* 2012). The Whatman no.1 filter paper has a thickness of 180 μm and a basis weight of 87 g m^{-2}, which corresponds to a density of ρ = 483 kg m^{-3} (Martinez *et al.* 2010; Busa *et al.* 2016; Yang *et al.* 2016). It has most frequently been the paper platform of choice due to the medium retention and flow rate. It is therefore referred to as the standard grade chromatographic paper (Liana *et al.* 2012; Cai *et al.* 2014). Whatman chromatography paper grade 1 was the first type to be utilized in 2007 and it has been subsequently used in many reported μPAD fabrication and detection methods (Blankenstein 2000; Martinez *et al.* 2007; Li *et al.* 2008; Yu *et al.* 2011; Yamada *et al.* 2015).

Whatman no.4 chromatographic paper has a larger pore size of 20 – 25 μM thereby increasing the surface area of the chromatographic paper and thus has a faster flow rate than the Whatman no.1 (Martinez *et al.* 2008; Li *et al.* 2010). Flow rate is defined as the migration speed of a fluid along the length of a strip and is one of the main parameters to take into consideration when choosing a paper type, alongside pore size and distribution (Songok and Toivakka 2016). It is the pore size of the paper which effects the retention time and the size of the particles which can be used on a particular paper platform (Lu *et al.* 2010). More so, the unique properties of paper which allow passive liquid transport and compatibility with chemicals/biochemicals are the main advantages of using paper as a sensing platform (Liana *et al.* 2012; Tenda *et al.* 2016).

2.10 Microfluidic patterning of a chromatographic paper

In fabricating paper devices, the choice of techniques and materials that meet the criteria of low cost, simplicity and efficient production process needs to be considered. Patterned paper has provided a new inexpensive platform for portable assays, which is simpler than the traditional microfluidic devices that are fabricated by etching or molding channels into glass, silicone, PDMS, or other polymers (Bruzewicz, Reches and Whitesides 2008; Cardoso *et al.* 2017). This process consists of the patterning of hydrophobic barriers onto paper that are able to confine an aqueous solution (Schilling *et al.* 2012). The idea of using paper as a platform for microfluidic devices takes advantage of the natural ability of the paper to wick aqueous solutions (Govindasamy *et al.* 2012; Li, Ballerini and Shen 2012; Xia, Si and Li 2016).

However, usually this ability cannot be controlled and fluid will wick in every direction as there are no barriers to restrict the fluid flow. Researchers have shown that barriers can be created in paper by treating the paper and making areas hydrophobic whilst keeping certain areas hydrophilic (as a channel) steering fluid towards a specific area or detection zone (Li *et al.* 2010). Since the initial idea of creating channels into a paper substrate, there have been many variations in the fabrication process of how this could be ideally completed (Ballerini, Li and Shen 2012). The most commonly used are wax printing, photolithography and screen printing (Dungchai, Chailapakul and Henry 2011). As a consequent, the main priority of each of these methods is to pattern a hydrophilic-hydrophobic contrast channel, which defines liquid penetration pathways into the paper either physically or chemically (Yetisen, Akram and Lowe 2013). Accordingly, microfluidic paper-based analytical devices permit the development of inexpensive analytical devices through the fabrication of hydrophobic patterns on chromatography paper (Martinez *et al.* 2010). The techniques for fabricating µPADs are listed in Table 2.1. Also, different patterning agents and the advantages as well as drawbacks of each technique are given. Different methods use different patterning hydrophobic agents and the patterning process defines the width and length of the channels while the thickness of the paper defines the height of the channel (Martinez *et al.* 2010). The characteristics of the paper influence the rate of the wicking, which also depends on temperature and relative humidity (Li, Ballerini and Shen 2012).

Table 2.1: Benefits and drawbacks of fabrication techniques

Fabrication	**Benefit**	**Drawback**	**Reference**
Photolithography	Can pattern an extensive variation of papers.	Requires a multistep process and sizable equipment.	(Martinez *et al.* 2008)
Ink Jet Etching	Requires a single printing apparatus to create channels by etching and to print bio/chemical sensing reagents.	Printing apparatus must be customized; thus it is not suitable for mass fabrication.	(Abe, Suzuki and Citterio 2008)
Analogue plotting	Can work with any surface (flexible device).	Inconsistent control over hydrophobic barrier formation.	(Bruzewicz, Reches and Whitesides 2008)
Wax printing	Produces enormous amount of devices with simple and fast fabrication process.	Requires expensive wax printer. Extra heating step is applied after wax deposition.	(Carrilho, Martinez and Whitesides 2009; Lu *et al.* 2009)
Plasma treatment	Useful for labs with a plasma cleaner wishing to make many replicates of a few sample patterns.	Requires different metal masks for different microfluidic pattern.	(Li *et al.* 2008)
Inkjet printing	Produces massive amount of devices with simple and fast fabrication process.	Has nozzle clogging. Requires extra heating.	(Li *et al.* 2010; Xia, Si and Li 2016)
Flexography	Low ink usage	Requires different printing plates and it printing	(Olkkonen, Lehtinen

	compatible with large scale production	quality relies on the smoothness of the paper	and Erho 2010)
Cutting	No contamination from chemicals and can fabricate 3D structures from paper and tape.	Manual method can result in low resolution and cannot produce arrays of free-standing hydrophobic patterns.	(Thuo *et al.* 2014)
Screen printing	No heat treatment and thus deposition of thick layer is possible.	Requires different printing screens for creating different patterns.	(Dungchai, Chailapakul and Henry 2011)
Laser treatment	Has high resolution.	Requires specialized laser equipment and has channels the do not allow lateral flow of liquids.	(Xia, Si and Li 2016)

Among the various techniques given in Table 2.1, wax printing is regarded as one of the most effective ways to produce µPADs due to the ease of its application and minimal instrumentation (Namwong *et al.* 2018). Wax printing requires a printer which is commercially available and a laminator. Wax printing is a rapid and inexpensive process. It is well-suited for producing large quantities (hundreds to thousands) of prototype µPADs with moderate resolution. Fabrication with wax is performed by penetrating wax through the paper depth to define hydrophilic microchannels on the paper substrate (Carrilho, Martinez and Whitesides 2009; Lu *et al.* 2009).

2.11 Methods for analyte detection on µPAD

Paper-based analytical devices offer the possibility to simple produce, mass-scalable devices at an affordable price, but it is also necessary to create an analytical device with suitable transduction methods. Currently, a variety of detection methods can be applied to µPAD that are compatible with their simplicity, affordability and portability. Detection is most commonly performed using colorimetric schemes, however, electrochemical; chemiluminescence and fluorescence techniques can also be used. The benefits and drawbacks of these methods are listed in Table 2.2 below.

2.12 Lateral flow on µPAD

The Whiteside’s group has recently created a complete new paper-based scrutiny that is based on lateral flow and has the ability to detect multiple samples in parallel and in a relatively short period of time onto the same device (Martinez *et al.* 2007). These devices are based on lateral flow of a fluid that is introduced onto one end or the center of the device and moves by the action of capillary forces. Capillary flow rate is a key parameter of µPADs and is defined as the migration speed of a sample front moving along the length of the test strip (Yetisen, Akram and Lowe 2013). Lateral flow offers advantages that are unique to paper and similar porous substrates (Pelton 2009). Intuitively, characterizing analyte flow through paper is challenging because paper is a complex medium, however, an understanding of the parameters that influence fluid flow in paper will enhance its application as a platform for analytical chemistry in paper-based microfluidic devices. Parameters of interest are the temperature and humidity (Camplisson *et al.* 2015), because paper-based microfluidic devices are intended for use in low-resource areas that can be located in a variety of climate zones. The geometry of a paper is also of interest in the determination of the variables that can be used to manipulate the analyte flow in the device, and the variables that offer design flexibility (Fu *et al.* 2011; Songok and Toivakka 2016).

Table 2.2: Benefits and drawbacks of methods used for analyte detection on µPADs

Detection method	**Benefits**	**Drawbacks**	**Reference**
Colometric	Simplicity	Judgment of final color can be challenging. Influenced by background noise of the paper.	(Schilling *et al.* 2012; Bhakta *et al.* 2014)
Electrochemical	Fast sensor responses, higher sensitivity (nM range) and independent of ambient light. Less prone to interference from the color/deterioration of the paper.	Requires reading equipment, increasing complexity and cost per test.	(Dungchai, Chailapakul and Henry 2009)
Chemiluminiscence	Based on the emission of light by a chemical reaction and has increased specificity and sensitivity.	Requires darkness or reading equipment making interpretation of results more complicated.	(Yu *et al.* 2011)
Flourescence	Increased specificity and sensitivity.	Scattering of light on the cellulose fibers. Influence of index refraction between cellulose and air. High cost.	(Taudte *et al.* 2013)

Among these detection methods, the colorimetric assay has proven to be most popular method in chemistry, particularly in combination with image collection via digital or smartphone cameras and analysis via available software for free download or even with naked eyes (Shen, Hagen and Papautsky 2012). Colorimetric detection is a well-established, fundamental method that has been the most adopted sensing mechanism for paper microfluidics (Yetisen, Akram and Lowe 2013). It provides easy readouts of the generated chemical signals, opening the door for instrument free measurements. Meticulous and carefully controlled measurements are required for quantitative analyses on µPAD in order to obtain accurate and precise analyte levels. Using physical and chemical properties of the chosen reagents and analytes interact to produce a visible color change when the analyte of interest is present. Accordingly, the change in colour can be observed by the users eye, or be measured via an image using computer software i.e. Adobe photoshop and ImageJ. However, these assays are subject to; varying light conditions, the user's interpretation and contamination from the environment, but can be controlled by ensuring identical parameters for each device and assay performed (Shen, Hagen and Papautsky 2012; Lopez-Ruiz *et al.* 2014).

In order to make the µPAD suitable for using in remote locations, not only the fabrication, but also the detection methods need to be affordable and cost effective. In this study, colorimetric method was used. This assay is simple, yet effective and is the most widely used analytical method performed on a paper device. This method typically involves a colour change reaction that can be observed by a naked eye (Li, Ballerini and Shen 2012). Currently, the analytes can be detected using colorimetric assay in the µPAD application by visually observing change in colour, intensity or brightness caused by the presence of the analyte.

Colorimetric assays of analytes using µPADs are possible by reflectance detection when the intensity of the colour that develops in the test zones is a function of the concentration of the analyte. Although the colorimetric approach do not require additional instrumentation, they are not as accurate compared to techniques such as UV-visible spectroscopy and atomic absorption spectroscopy. This is mainly because the colour and intensity interpretation by eye is different for each individual, ambient light condition, as well as the condition of the paper substrate (dry or wet). Thus, in order to improve accuracy and sensitivity of the method, Martinez an co-workers raised that apart from measuring the change in colour by the naked eye, it can also be captured by a desktop scanner or a digital camera (Martinez *et al.* 2009). The image can then be transferred to a computer and the colour can be measured or interpreted using computer software i.e. Adobe Photoshop and ImageJ (Ellerbee *et al.* 2009; Cate *et al.* 2013). Additionally, it has been pointed out that colorimetric assays are regarded to be suitable for analytes detection since (i) the colour change can easily be observed by naked eye; (ii) they use readily available reagents; (iii) they can be used on different conditions of the environment; (iv) appropriate assays provide sensitivity to specific analytes; (v) they produce results quickly; and (vi) they can be easily interpreted by semiskilled technicians (Ellerbee *et al.* 2009; Tenda *et al.* 2016)

CHAPTER THREE

RESEARCH METHODOLOGY AND DESIGN

3.1 The research design

A quantitative research methodology was chosen for this study. Consequently, Onwuegbuzie and Leech elucidated that quantitative research entangle identifying the aspect of an observed case, or describing or exploring possible correlations among two or more cases (Onwuegbuzie and Leech 2007). Hence, the quantitative approach was used to quantify the problem by way of generating numerical data or data that can be transformed into useful statistics. The collection of data can be achieved either through direct observation or through gathering from records. Thus, the assembled data was entered into a computer where it was counted, stored and manipulated and was used to quantify attitudes, opinions, behaviors, and other defined variables (Wiersma and Jurs 2005; Hopkins 2008).

The quantitative research methodology elucidates the techniques used by the researcher to present, analyse and discuss the statistics and correlations. In affirmation of the actual constituent of the research, it is important to know these five W’s, **Who** (organization, company, person/people), **When** (Month, year or day), **Where** (Place, point, site, position, spot or venue), **Why** (necessity of conducting the research) and **Way** (method or technique). This study answers sought (captured in brackets) as follows:

Who? (ETP dam located within The Umbogintwini Industrial Complex);

When? (During Month of September 2017);

Where? (The Umbogintwini Industrial Complex);

Why? (To determine the concentration of ammonia present in the ETP wastewater dam before disposal), and

Way? (Use of a paper-based microfluidic analytical device).

The fundamental approach to the study was a formal, objective and methodical process in which numerical data was sourced through ImageJ software and the data utilized to plot graphs and calculate the quantity of ammonia present in ETP wastewater Dam.

3.2 Target population

The target population comprised of companies located within the Umbogintwini Industrial Complex, Durban, KwaZulu-Natal, South Africa, as illustrated in **Fig 3.1**. The sampling points were designed in relation to Effluent plant treatment Dam at Acacia.

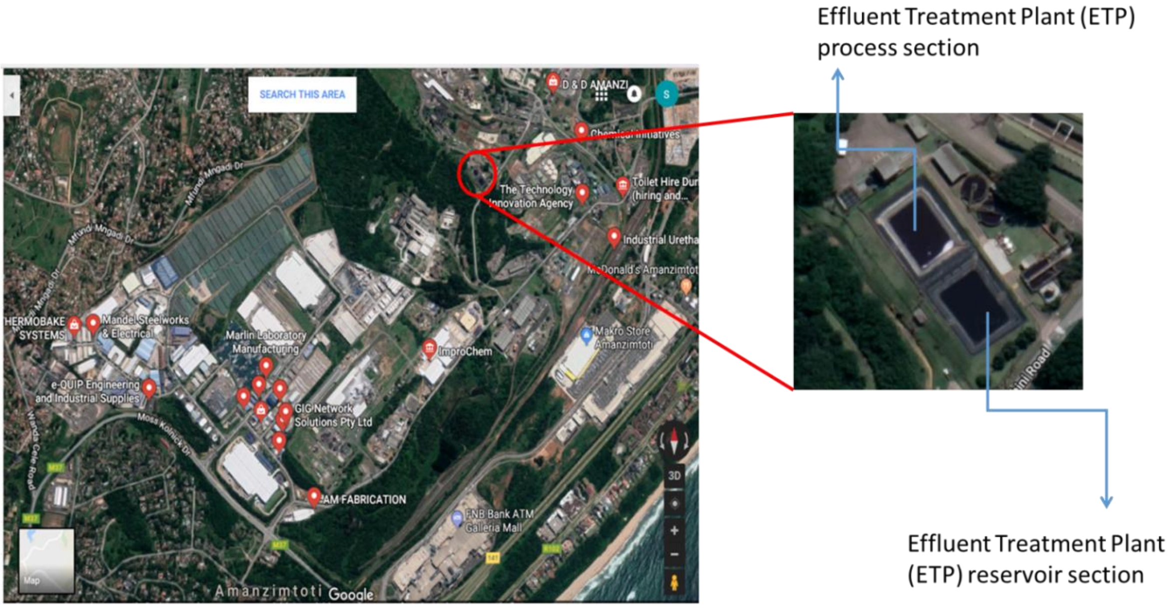


Figure 3.1 ***Map of the study area showing the location of the sampling site***

In the ETP process section; it is where the treatment takes place and the ETP reservoir section; it is where the wastewater is ready to be released to the sea. The UIC is home to many large recognized industries, currently 11 major companies are operating. These companies together with their activities are listed in Table 3.1.

Table 3.1 Industries evaluated and their individual production activities

Industry	Activities
Dulux	Paint
Resinkem	Fabricates and markets urea formaldehyde resins, formaldehyde solutions, urea, and resins for the board, timber, paper, creature feed and foundry enterprises.
Dyefin textile	Specialising in dying, finishing and print services to the textile industry.
Evonik Industries	Manufactures and distributors of hydrogen peroxide.
Improchem	Gives vital solutions, water treatment, water optimisation and total water management to industry and water expert in Southern Africa.
KK animal nutrition	Supplier's phosphate sector through acquisition joint, ventures and organic growth.
Lake IT	Manufacturer and distributor of products and services for explosives, fertilizers, food ingredients, coatings, and general chemicals.
Chemical Initiatives	Producer and provide of sulphur-based chemicals and services to sectors such as agriculture, mining, paper and packaging.
Anchor yeast	Specialise in yeast and bacteria.
Acacia (heartland)	Supplier's portable water, steam, electricity, laboratory facilities, security, rail, engineering workshops.

Some of these industries use ammonia in their products and process. However, for most industries, ammonia is a waste product requiring safe disposal. The pollution inventory includes all the entities that discharge quantities of pollution significant enough to warrant monitoring. Industrial point sources of ammonia discharge are entities that produce sizable quantities ammonia impaired wastewater that requires treatment before being carried over to the sea and assurity that they fall within the specifications placed by the Department of Water Affairs (DWA). Most industries produce nominal amounts of ammonia dissolved in wastewater, but some industries produce significantly larger amounts and concentrations of ammonia wastewater.

3.3 Sampling

Effluent samples were collected from the ETP dam for ten operational working days. The samples were collected as a composite sample, meaning each sample is a mixture of samples collected in three intervals, from morning, evening and midnight. These samples were collected in the month of September in 2017, since this is mainly the busiest of the business in all industries based in the complex due to the coal and steam demand for the month of December break. All samples for laboratory analysis were placed into thoroughly cleaned (cleaned with dilute nitric acid and rinsed with distilled water before use) 1 L plastic bottles and tightly closed. Each bottle was rinsed with the appropriate amount of sample prior to sample collection. These samples were placed in a cooler box and protected from direct sunlight and then taken to the laboratory and filtered.

3.4 Materials and methods

3.4.1 Chemicals and apparatus

Mercury (II) chloride (99%), potassium sodium tartrate and sodium nitroferricyanide (99%) were purchased from Sigma-Aldrich (Steinheim, Germany). Potassium iodide, sodium hydroxide (99%), sodium salicylate and ammonium chloride were purchased from Merck (Darmstadt, Germany). A Cary 50 Conc UV-Visible spectrophotometer was purchased from Varian Instruments PTY(LTD) (Cape town, Western Cape), Samsung galaxy J1 purchased from Game (Durban, South Africa), a Xerox ColorQube 8570 wax printer purchased from Xerox (Norwalk, CT, USA).

3.4.2 ImageJ analysis

ImageJ was used to calculate the RGB (red, green, blue) colour system and the mean intensity measured for each standard/sample concentration. The colour information is mainly obtained as separate RGB channel values or grayscale (intensity), which give the amount of analyte on the basis of the relationship between the colour information and the analyte concentration (Soldat, Barak and Lepore 2009). A smartphone was used to take images of the observed colour change. These images were then transferred into a computer for Image analyses using Image J software and were statistically measured. The performance of the system was assessed by comparing it with the standards performed using UV Visible, and then determining any statistical difference within the results achieved on the paper device.

3.4.2.1 The CIELAB Procedure

The difference in colour was quantified relative to a blank sample. The formula for colour difference calculation is depicted in equation 3.1.

$$\Delta \mathrm{E} = \sqrt{(\Delta \mathrm{L}*)^2 + (\Delta \mathrm{a}*)^2 + (\Delta \mathrm{b}*)^2} \tag{3.1}$$

where ΔE is the colour difference, and ΔL*, Δa* and Δb* are the respective differences between the measured and predicted CIELAB values (Kasson *et al.* 1995; Hunt and Pointer 2011). The CIELAB colour system was utilized and the colour difference equation (ΔE) was used to colorimetrically detect the levels of ammonia in wastewater. If colors are represented by the CIELAB space, the axes of lightness L* and chromaticities a* and b* have to be suitably quantized. Therefore, the range of colors and the limitations of the axes have to be specified. The standardized CIELAB definition gives a limit only for the L* axis ($0 < L^* < 100$) whereas no limitations are specified for a* and b* (Sharma, Wu and Dalal 2005).

3.4.2.2 CIELAB parameters calculation

The CIELAB values, XYZ colorimetric parameters of all samples were obtained by transforming the resulting RGB signals to CIE tristimulus values as follows (Zhang and Wandell 1996):

$$X = 0.4303R \ + 0.3416G + 0.1784B$$

$$Y = 0.2219R \ + 0.7068G + 0.0713B$$

$$Z = 0.0202R \ + 0.1296G + 0.9393B$$

CIELAB parameters, in contrast to tristimulus values, enables ready comprehension of the visual attributes of a colour. The way in which L *, a*, and b* relate to the actual experience of seeing colours varies. The mathematical transformations of tristimulus values to CIELAB coordinates were developed by trial and error. They are L*a*b* values (Hunt and Pointer 2011):

$$L^* = 116\ (Y/Y_n)^{1/3} - 16$$

$$a^* = 500[f(X/X_n) - f(Y/Y_n)$$

$$b^* = 200[f(Y/Y_n) - f(Z/Z_n)$$

where:

$$f(X/X_n) \ = \ (X/X_n)^{1/3}$$

$$f(Y/Y_n) \ = \ (Y/Y_n)^{1/3}$$

$$f(Z/Z_n) \ = \ (Z/Z_n)^{1/3}$$

X_n, Y_n and Z_n are the tristimulus values for a particular standard illuminant and observer. Their values are available in many standard texts and are incorporated in the software of modern colour-measuring. As a result, the recognized deviations of CIELAB space from perceptual uniformity, other transformations of tristimulus values are available for the specification of colour differences, but with a concomitant loss of the conceptual simplicity of CIELAB space.

3.4.3 Prepared reagents

3.4.3.1 Nessler's reagent

Nessler reagent was prepared by dissolving 4.4 g of mercury (II) chloride in 40 mL distilled water. Complete dissolution was achieved by heating the solution on the hot plate. In a separate flask, 11.0 g of potassium iodide was dissolved in minimum water possible (approximately 2 mL). This saturated solution of potassium iodide (KI) was then added drop wise into the prepared solution of mercury (II) chloride. This resulted in abundant precipitation of orange mercury (II) Iodide (HgI_2). A 100 mL solution of 5 M sodium hydroxide was prepared by dissolving 20 g of sodium hydroxide in sufficient water. Thereafter, 40 mL of the prepared sodium hydroxide solution was transferred into the complex solution in a 250 mL beaker.

3.4.3.2 Salicylate reagent

This was prepared by placing 10 g of sodium salicylate in a volumetric flask which was then filled with 70 mL of deionized water. Thereafter, 0.04 g of sodium nitroferricyanide was added. The resulting solution was thoroughly mixed until all solid materials dissolved. This was followed by the addition of 0.5 g of sodium hydroxide in order to adjust the pH to approximately ~12 (Verdouw, Van Echteld and Dekkers 1978).

3.4.3.3 Hypochlorite solution

A volume of 1.0 mL for bleach was placed into a 100 ml volumetric flask, followed by the addition of 70 mL of deionized water. Thereafter, 0.5 g of sodium hydroxide was added and the resulting solution was thoroughly mixed until dissolution was achieved. Thereafter, the flask was filled with deionized water to the 100 mL calibration mark.

3.4.3.4 Ammonia standards

A stock solution containing 100 mg L^{-1} of ammonia was prepared by dissolving 0.3818 g of ammonium chloride in distilled water and diluting to 1000 mL with deionized water. Standard solutions in the concentration range of 0-5 mg L^{-1} of ammonia were prepared by suitable dilutions of the prepared stock solution.

3.4.4 Calibration and Fabrication

3.4.4.1 Xerox Wax Printer

Xerox printer was used to print microfluidic shapes designed in Adobe Illustrator CC software (Yetisen, Akram and Lowe 2013). The design or patterning of the microfluidic paper-based analytical device was developed using the graphic software on a computer then printed on a suitable paper using wax printing. Predominantly, indistinguishable designs of wax were printed in arrangement of the Whatman No.1 chromatography paper substrate cut into A4 size before used. The sheets of Whatman No.1 chromatography paper were directly fed into the printer. The print head dispenses ink (melted wax) on the surface of the paper, where it cools and solidifies immediately without spreading further. The ink is consist of a mixture of hydrophobic compounds including carbamates, hydrocarbons and dyes that melts around 120 ºC. The µPAD were produced by the wax pattern manufacturing process. The wax patterns were designed using the CorelDraw X7 software, followed by wax printing on chromatographic paper using a wax printer (Xerox Colorqube 8570) shown in **Fig 3.2**. The printed paper was calcined at 150 °C for 2 min and was cooled to room temperature.

Figure 3.2 *Xerox Colorqube 8570*

3.4.4.2 UV Visible spectrophotometer

A single beam ultraviolet- visible spectrophotometer (model Varian-Cary Win UV50) was employed to validate and verify the colorimetric effect of ammonia on the paper based microfluidic device since it is already an established method. It is therefore a suitable method for validation of colorimetric sensors. This method was employed for all direct measurements. The instrument parameters were controlled by the Cary-Win software (Varian). Data were collected and processed by the software as well.

3.4.4.3 Scanning electron microscopy (SEM)

The samples surface topography of the chromatographic paper was characterized using a field-emission scanning electron microscope (SEM) (EVO 15 HD, Carl Zeiss) with an accelerating voltage of 3 kV. Each sample was sputter coated with an ultra-thin layer of gold to prevent charging of the paper samples during imaging. To obtain a visual depiction on the microscale of the fibrous medium being investigated, SEM was used to take micrograph images of the chromatographic paper before and after heating in order to gain a physical understanding of pore and fibre structure of paper, and how it may influence flow. A square piece of 5 mm was cut from each type of paper and placed on an aluminum stub 9.5 mm in diameter and 9.5 mm in height, using double sided carbon tape to eliminate interference from the aluminum stub. Those were coated with gold under vacuum in an argon atmosphere.

CHAPTER FOUR

PRESENTATION ANALYSIS AND DISCUSSION OF RESULT

4.1 Calibration and fabrication of the microfluidic paper-based analytical device

4.1.1 Xerox wax printer

The wax printer in **Fig 4.1(a)** was used to print out the patterns on the chromatographic paper shown in **Fig 4.1(b)**. The printed paper was thermally treated at 150 ºC for 2 min and then cooled to room temperature so that the wax melted and penetrated through the paper to form hydrophobic wax barriers on paper as shown in **Fig 4.1 (c)**.

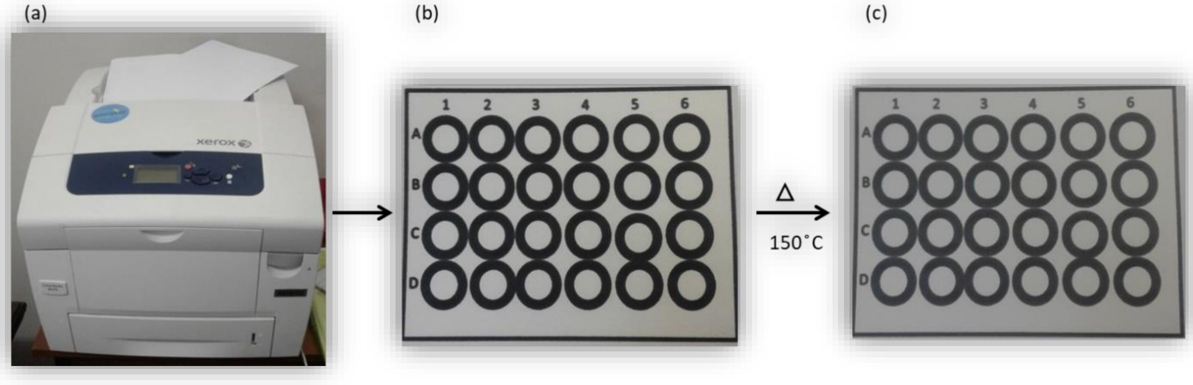


Figure 4.1 *Fabricated µPAD (a) Xerox wax printer used to print A4 size of the fabricated chromatographic paper, (b) printed chromatographic paper, (c) chromatographic paper heated at 150ºC to produce hydrophobic wax barriers*

4.1.2 Scanning Electron Microscope (SEM) Analysis

Scanning electron microscopy (SEM) was used to understand whether heating the µPADs affected the basic morphology of the paper affected the basic morphology of the paper. The µPADs were studied at the coveted magnification for the morphology of the particles as shown in **Fig 4.2** and **Fig 4.3**.

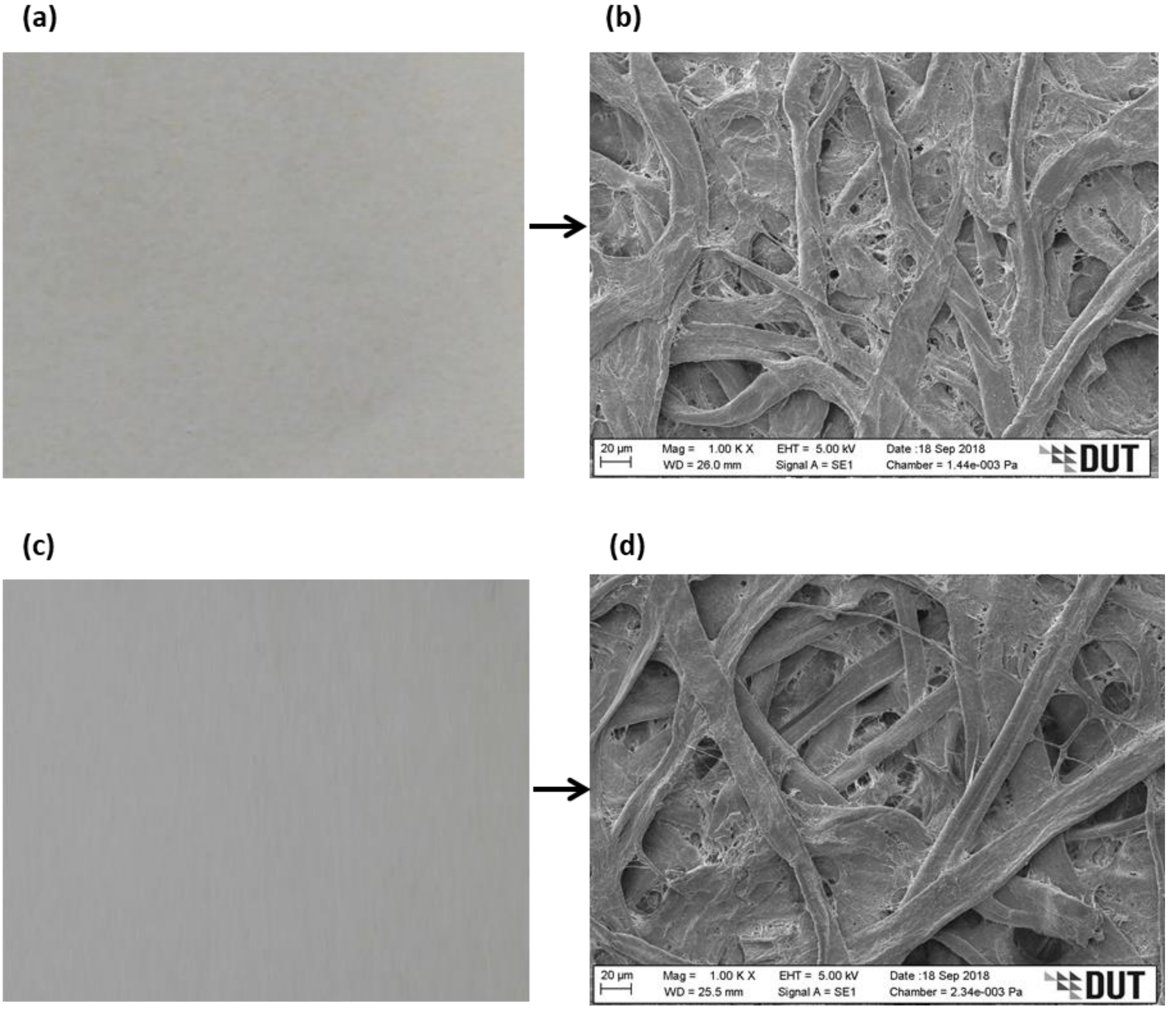


Figure 4.2: *SEM micrographs of (a) plain PAD before heat with it SEM micrograph (b) while (c) is the plain PAD after heat with it SEM micrograph at (d), which was done at a low voltage of 3.0 kV and a magnification of 1000x*

The images of the µPAD before heat **(Fig 4.2 (a))** and after heat **(Fig 4.2 (c))**, showed no significant difference. A close look at the fibre structure of the chromatographic paper demonstrates that interfibre pores were made by the spaces between filaments, which tend to be highly inconsistent in size. The fiber organization of the paper after heating in **Fig 4.2 (d)** showed that the paper after heat **(Fig 4.2 (c))** was normally kept, i.e. there were no noticeable structural differences in fiber organization between the paper before heating and after heat treatment at 150 ºC.

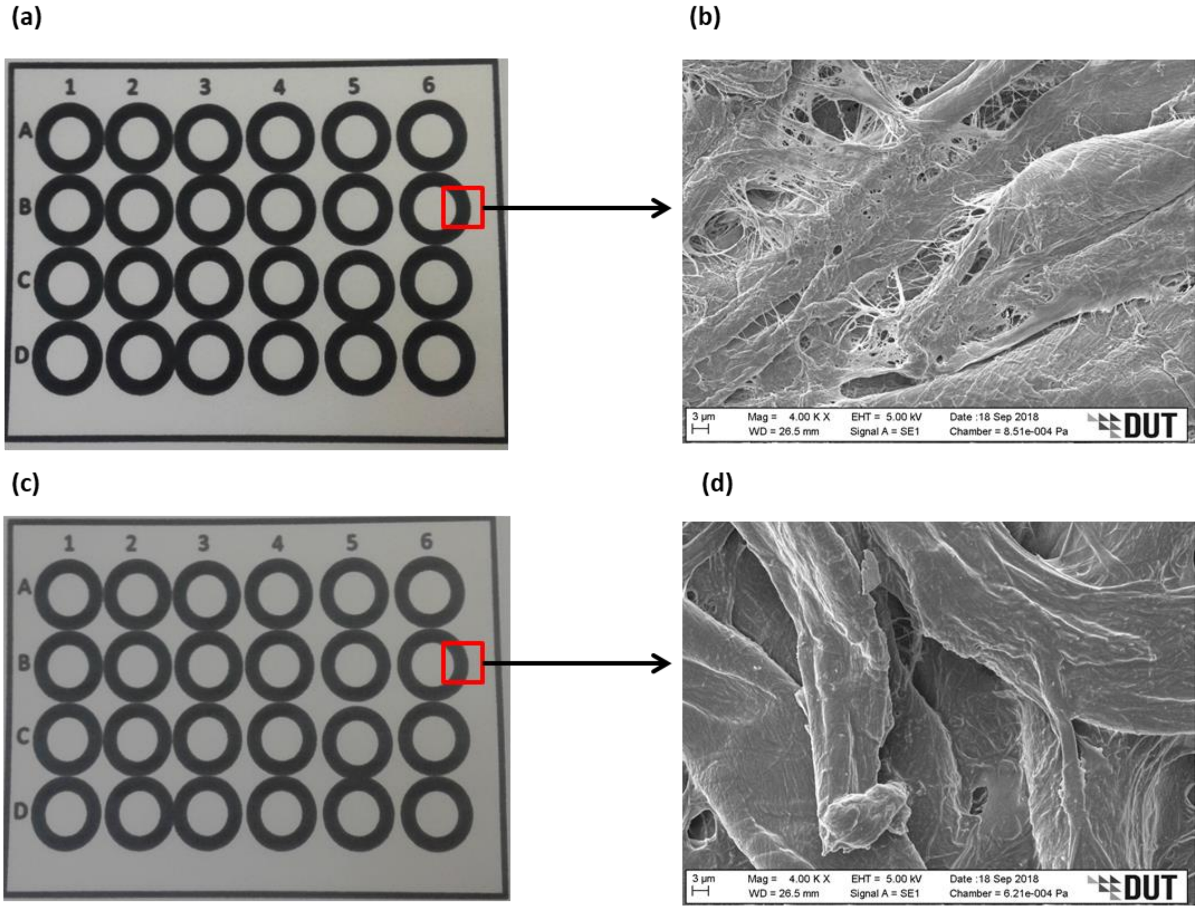


***Figure 4.3**: Schematic representation of µPADs where (a) is a schematic illustration of general structure of wax-printed µPAD and methodology used to detect ammonia before heat. (b) SEM image of the black line before heat. (c) The black line was melted on a hot plate and (d) the SEM of the melted black line.*

The µPAD images in **Fig 4.3 (a)** showed a hydrophilic black line of wax on a white background subsequently, while in **Fig 4.3 (c)** is a hydrophobic black line (wax) melted on a hot plate. A closer look at the micrographs in **Fig. 4.3 (c)** and **(d)** above showed some interesting differences in the structure of the fibres themselves. It was noticed that, the large paper fibres made up of smaller fibres intertwined, and fibres as small as 10 nm in diameter were detected in the micrograph of the wax before heating (b), while after heating (d), the smaller fibres interwined were reduced. This observation in fibre structure reiterated the complexity of the fibre network structure of paper, and emphasized the complication involved in assigning a representative average pore size.

4.1.3 UV Visible spectrophotometer

For all NH_3 standard solutions, the UV visible spectrophotometer was blanked with Deionised water prior to the measurements. This approach was used to validate the results obtained using a microfluidic paper-based analytical device. The validation was also done for both, Nessler reagent and salicylate method. The product from the reaction between the Nessler reagent and aqueous ammonia absorbs light over a wide wavelength range, that is, 400 nm – 425 nm (yellow brownish-coloured light) (Meseguer-Lloret, Molins-Legua and Campins-Falco 2002). Whereas, the Salicylate method for the direct determination of ammonia was utilized due to its high sensitivity at the mg L^{-1} level and intense colour development in the visible region at 650-660 nm (green colour). The results for the absorbance values obtained from each method are displayed in Table 4.1.

Table 4.1 Spectrophotometric optimization of ammonia standards

[NH_3] (ppm)	**Nessler reagent Method**	**Salicylate Method**
Absorbance	**@425nm**	**@630nm**
0	0,022	0.0074
0.1	0,039	0.074
0.5	0,086	0.127
1	0,145	0.257
3	0,314	0.544
5	0,500	0.911

From the results obtained in Table 4.1, a calibration curve of absorbance against concentration of ammonia was plotted. It was observed that the higher ammonia concentration results in more intense drop in the absorbance intensity of the solution at 425 nm for the Nessler reagent method. For the salicylate method, ammonia was reacted with hypochlorite and salicylate to give a green colour, yielding the absorbance intensity of the solution at 630 nm. The calibration curve of absorbance corresponding to each level of ammonia is shown in **Fig. 4.4**.

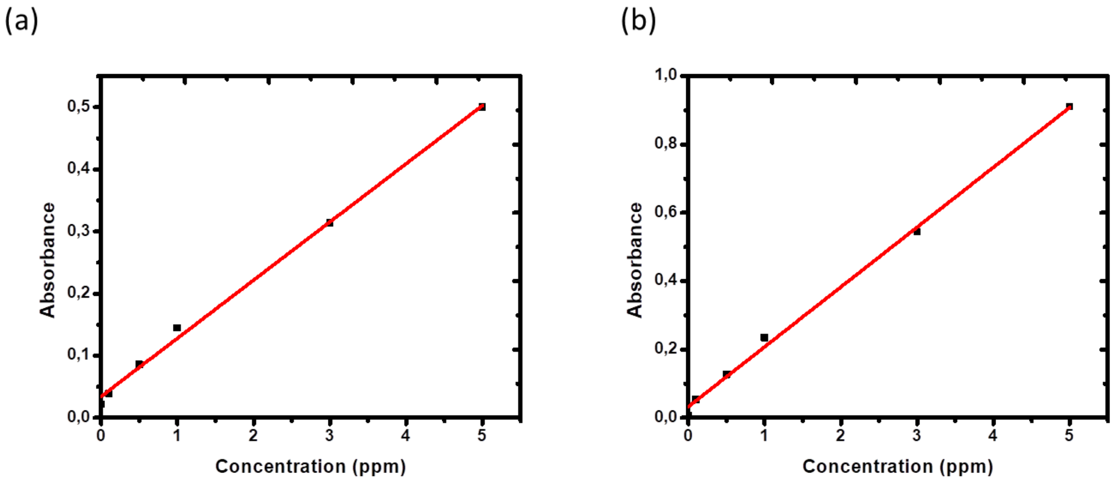


Figure 4.4; *UV–visible spectra of ammonia standards (range of 0-5 ppm) for (a) the Nessler reagent method and (b) salicylate method*

For both methods, the more intensely coloured solutions of ammonia standard with the reagent yielded a higher absorbance and in turn a higher apparent ammonia concentration. The R^2 values for the Nessler reagent method was 0.9964 and for the salicylate method was 0.9966. The absorbance values of the salicylate method were a little higher than that of Nessler reagent method. The reason of this occurrence is not clear yet, but the presence of mercury on the Nessler reagent method must have had an effect on the colour intensity. Other possible reasoning could be that, the salicylate method absorbs light at a higher wavelength than the Nessler reagent method.

4.2 Calibration of analytical methods

As confirmed by (Massart, Dijkstra and Kaufman 1978; Kuo and Zhu 2012), optimization testing helps to increase the reliability, validity and practicability of the analytical method. Essentially, optimization tests sharpen research procedures, clarify the permissions and approvals needed to conduct the study, provide indicative costs in time, and check the viability of the larger study.

The colorimetric method of detection is best suited for paper microfluidic devices which had constantly been upgraded, renewed and retested to allow for the most accurate method to be exploited (Martinez *et al.* 2010; Morbioli *et al.* 2017; Yakoh *et al.* 2018). This takes into account both quantitative analysis (information which can be estimated numerically) and subjective investigation (information which depends on perception and understanding) to be performed (Amaratunga *et al.* 2002). The use of digital results taken from many paper microfluidic devices already available allows a numerical value to be calculated from the result e.g. ImageJ provides a numerical measurement according to the intensity of a colour change thereby allowing for a quantitative result to be measured which is more accurate than qualitative measurements.

In order to quantify ammonia in wastewater, it is of great importance to seek for a practical method that is efficient and sensitive as much as possible. Although there are several colorimetric methods used for the determination of ammonia, these methods have their own characteristics, but each has its restriction, or has different levels of investment in equipment, high operating costs, secondary pollution, and other deficiencies.

In this research two colorimetric methods were used for the quantification of ammonia in wastewater. The first one was the Nessler method in which mercury (II) chloride and potassium iodide was reacted with NH_3 to create a yellow-brownish coloured $NH_2Hg_2I_3$ compound (Li-qiong and Yong 2011; Chruszcz-Lipska *et al.* 2015). The second one was the salicylate method where the hypochlorite and salicylate was reacted with NH_3 in the presence of a catalyst (nitroferricyanide) to create a blue coloured indosalicylate compound (Larsen, Dalsgaard and Pedersen

2015; Zhou and Boyd 2016). The colorimetric approach of these methods was validated using UV Visible spectrophotometer. The colorimetric approach of the microfluidic paper-based analytical device was analysed by the addition of standards with the reagent, the reaction between the two resulted in the change of colour which was captured by a smartphone, then transferred on a computer for image analysis **Fig. 4.5**. The performance of the system was assessed by comparing it with the spectrophotometric method, and then determining any statistical difference within the results achieved on the paper device. The data obtained was transferred to Origin Pro 8 and a linear regression fitted to the standard concentration. For the ETP wastewater samples, their concentrations were calculated using the linear equation from the regression.

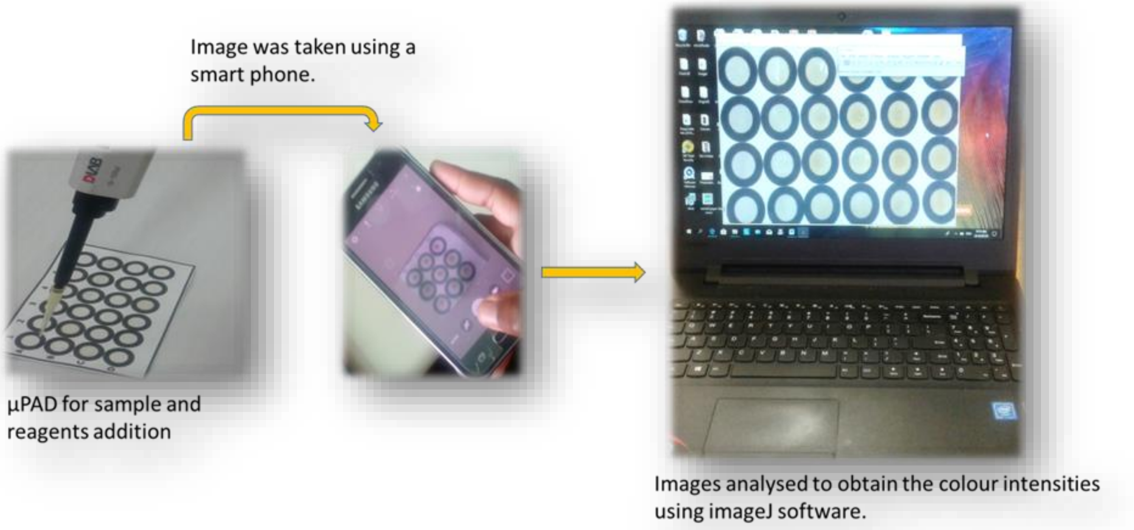


***Figure 4.5**: Illustration for the quantitative detection of ammonia using µPAD*

4.2.1 Colorimetric analysis of ammonia with Nessler reagent

Nessler's reagent – named after Julius Nessler, the German chemist who first made this reagent in 1856 - is a solution consisting of mercury (II) iodide and potassium iodide. It is a national standard and classical method for determination ammonia nitrogen in water (Thompson and Morrison 1951). The method has the advantages of easy operation and high sensitivity towards ammonia. With reference to equation 4.1, it can be observed that the, Nessler reagent (K_2HgI_4) reacts with the ammonia present in the sample under highly alkaline conditions to produce a yellow-brown colour complex ($NH_2Hg_2I_3$) when assessed colorimetrically (Demutskaya and Kalinichenko 2010; Wu and Cao 2013; Lin *et al.* 2014). The intensity of the colour is in direct proportion to the ammonia concentration. Rochelle salt [potassium sodium tartrate ($KNaC_4H_4O_6 \cdot 4H_2O$)] solution is normally added to remove residual hardness cations that might react with Nessler's reagent. The basic chemical equation for ammonia reaction with Nessler's reagent is:

$$\mathbf{2K_2HgI_4 + 2NH_3 \rightarrow NH_2Hg_2I_3 + NH_4I + 4KI} \qquad (4.1)$$

Ammonia is produced from the reduction of ammonium with potassium hydroxide. Reaction results in a colour change from pale yellow to brown. In this study, optimization of ammonia were achieved by dropping 10 µL of Nessler reagent and 5 µL of potassium sodium tatrate (Rochelle salt) into a reaction zone of the µPAD containing 20 µL of ammonia standards of different concentrations (0, 0.1, 0.5, 1, 3 to 5 mg L^{-1}). In this colorimetric test, the Nessler reagent and potassium sodium tatrate were added into the standards and a resultant change in colour was observed from pale yellow to brown as observed in the reported work by Wu and Cao (2013) (Wu and Cao 2013). As displayed in **Fig. 4.6**, sample spots were in yellow colour, and the colours gradually become deeper as the ammonia concentration was increased from 0.1 to 5 mg L^{-1}. The change in colour was observed after 1 minute, thereafter, the pictures were captured using Samsung galaxy J1 smart phone and processed with Image J software.

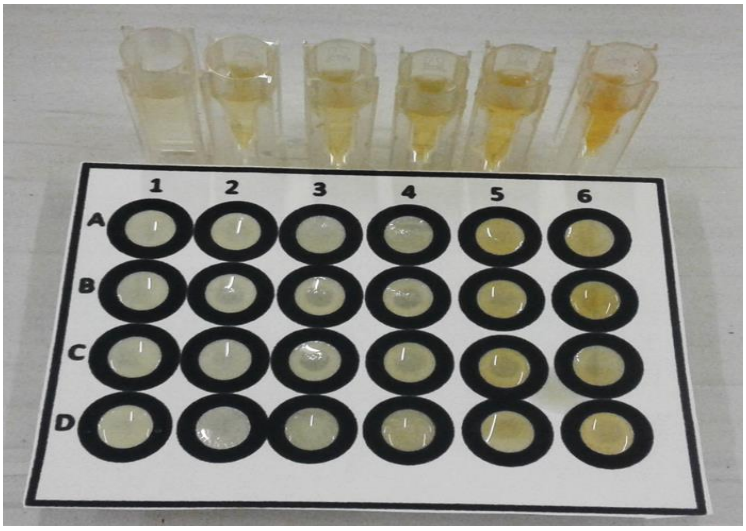


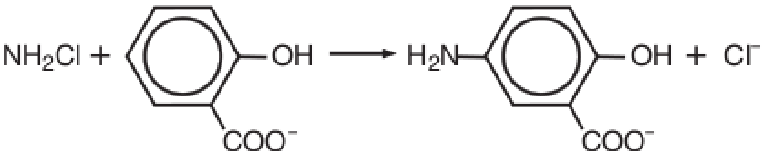
Figure 4.6 *Optimized colour change from the concentration range of 0-5mg L^{-1}*

The developed colours corresponding to various concentrations of ammonia are represented in **Fig 4.6**. It was observed that low ammonia concentrations resulted in the formation of light yellow colour on the µPAD. The yellow colour became more intense on higher ammonia concentrations. However, the colour variability among standards was not clear for direct observation, with the aid of imageJ intensity analysis, the small difference between all standards could be determined accurately as shown in Table 4.2.

4.2.2 Colorimetric analysis of ammonia with Salicylate method

The Salicylate method is a variation of the well-known Phenate method, but it has an advantage of being free from mercury salts and phenol (Weatherburn 1967; Bower and Holm-Hansen 1980). This method is reported to be most useful for low range ammonia determinations (Willason and Johnson 1986; Yang and Cheng 2007). However, it procedure involves multiple reactions before a final green colour is developed. The first reaction step involves the conversion of ammonia to monochloroamine by the addition of chlorine. The monochloroamine then reacts with salicylate to form 5-aminosalicylate. Oxidation of 5-aminosalicylate is carried out in the presence of a catalyst, sodium nitroprusside or $Na_2[Fe(CN)_5NO]$ (also called sodium nitroferricyanide), which results in the formation of indosalicylate, a blue-colored compound. The blue color is masked by the yellow color (from excess nitroprusside) yielding a green-colored solution. The intensity of the color is directly proportional to the ammonia concentration in the sample (Bower and Holm-Hansen 1980).

$$NH_3 + OCl^- \longrightarrow NH_2Cl + OH^- \qquad (4.2)$$



(4.3)

According to reaction equation 4.2 for ammonia test with Salicylate method, ammonia compounds are initially combined with hypochlorite to form monochloroamine which reacts with salicylate (as shown in equation 4.3) to form 5-aminosalicylate (Bower and Holm-Hansen 1980).

Applying the method on a µPAD, 10 µL of each standard was dropped in the detection zone followed by 2.5 µL of hypochlorite and salicylate catalyst. After 5 minutes, the colour of the detection zones gradually changed with the increase in concentration from yellow to dark green as shown in **Fig 4.7**. Thereafter an image was captured and processed for intensity.

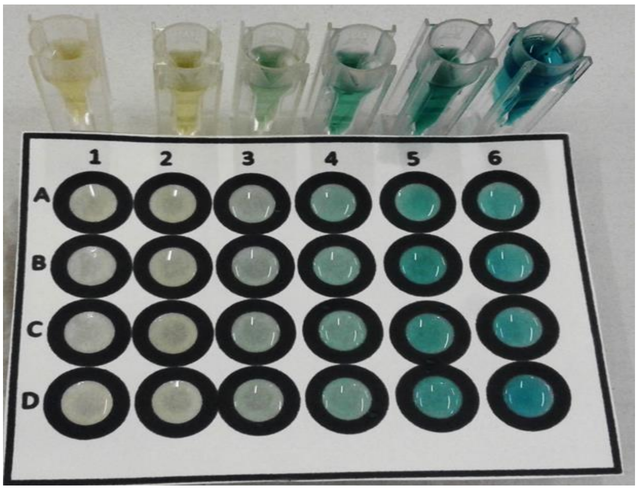


Figure 4.7 *Optimized colour change from the concentration range of 0-5 mg* L^{-1}

The colour intensity was obtained by drawing a 10 mm circle in each spot and measure analysis was selected from Image J toolbar. The colour information mainly obtained from the grayscale or intensity, gave the amount of analyte on the basis of the relationship between the colour information and the analyte concentration. The results obtained for the colour intensity are shown in Table 4.2.

Table 4.2 The quantified values of ammonia obtained from the Image J analysis for the Nessler's reagent and Salicylate method

Concentration (ppm)	**Intensity**	
	Nessler's reagent method	**Salicylate method**
Blank	144	107
0.1	155	115
0.5	163	122
1	165	129
3	175	135
5	178	142

The intensity for Nessler reagent method which was ranging from 144 to 178 was higher than that of the Salicylate method which was ranging from 107 to 142 as shown in Table 4.2. These two methods showed proportion of the intensity with concentration. Calibration curves of Nessler Reagent and salicylate methods in **Fig 4.8** showed that there was a relatively wide linear range for the detection of ammonia (0–5 mg L^{-1}). The quadruplicate analyses of water samples for precision estimate, Nessler method gave higher mean concentration of ammonia than the Salicylate method .

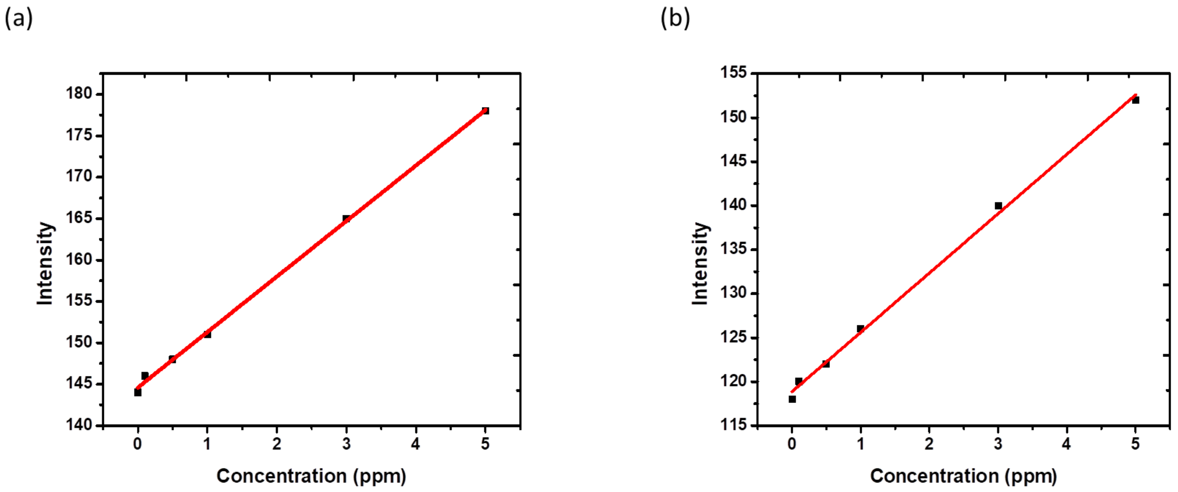


Figure 4.8 *The standard curves the intensity against the concentration of ammonia for both (a) Nessler reagent and (b) Salicylate method*

4.2.3 Comparison of methods

The limits of detection (LOD) and quantification (LOQ) were calculated from the relationship between the standard deviation (SD) of the Intensity and absorbance linearity and the slope, using the appropriate multiplier. These were calculated as $3S_b/m$ for LOD and $10S_b/m$ for LOQ, where, **S_b** is the standard deviation of the blank signals and **m** is the slope of the calibration curve (Shrivastava and Gupta 2011). Results for the developed microfluidic paper-based analytical devices for the determination of ammonia using both Nessler reagent and salicylate colorimetric methods are presented in Table 4.3.

Table 4.3: Analytical figures of merit

Parameters	**Colorimetric approach**		**Spectrophotometric**	
	Nessler reagent	**Salicylate**	**Nessler reagent**	**Salicylate**
Accuracy	1.92±2.05	1.92±2.05	1.92±2.05	1.92±2.05
Slope	5.8	6.7	0.094	0.173
Intercept	149.8	108.5	0.034	0.043
Linearity	0 – 5	0 – 5	0 – 5	0 – 5
Correlation coefficient	0.9644	0.9993	0.9964	0.9966
LOD/ units	3.37	3.20	0.52	0.75
LOQ/ units	10.2	9.68	1.52	2.27

Accuracy=mean ± standard deviation

The colorimetric and spectrophotometric approaches for both the Nessler reagent and Salicylate methods were used to evaluate the applicability of the developed µPAD for determination of ammonia. For the colorimetric detection of ammonia using Nessler reagent, the LOD and LOQ were 3.37 and 10.2, whereas for the spectrophotometric approach was 0.52 and 1.52, respectively. While the colorimetric detection of ammonia with salicylate had LOD and LOQ of 3.20 and 9.68, and for spectrophotometric was 0.75 and 2.27, respectively. The determined spectrophotometric LOD and LOQ gave different results from those observed calorimetrically. These differences suggested by linear regression from linearity curve provided a satisfactory result, where the standard deviation of the y intercept was reduced; this was directly proportional to both limits. The experimental determination could have been affected by other interfering factors, such as human manipulation and differences in the standard conditions among laboratories (Martinez *et al.* 2008; Jayawardane, McKelvie and Kolev 2015).

4.3 Colour measurements

In order to assess the possibility to use the device outside a laboratory setting, digital images of the paper devices were taken using a Smartphone. The use of a smartphone would allow the user to be less reliant on expensive equipment and established laboratory to complete the analysis (Moonrungsee, Pencharee and Jakmunee 2015). For example, to be able to use the paper device in the field, it images could be sent via email to a central laboratory and analysed on the ImageJ software. An application (app) can be created to analyse the data from the paper device by a mobile phone (Shen, Hagen and Papautsky 2012; Lopez-Ruiz *et al.* 2014). In this case, images once captured were transferred to a computer and analysed with ImageJ.

4.3.1 RGB colour space

RGB is an additive colour space and is mainly used throughout computer graphics as colour displays usually apply red, green, and blue to create desired colours (Connolly and Fleiss 1997). The main idea for the development of the RGB colour space was simulating the human visual system. This model decomposes the colour of an image into three components (red, green and blue) and was used to calculate the respective intensity of these three components in the image. Each of three primary colours has a pixel ranging from 0 to 255 (Moonrungsee, Pencharee and Jakmunee 2015). The colour information obtained from the sensing area was used for evaluating the performance of the µPADs. In this way, the colour change of wastewater samples upon the addition of reagents and the relation between colour change and the samples concentration were quantized.

4.3.1.1 Nessler reagent method: Extraction of the RGB values

The analysis of the RGB colour intensity was done using the image in **Fig 4.6**. Snapshots of the colour histogram for each standard are shown in **Annexure 1**.

Each value indicated the maximum pixel colour intensity (the value of the rightmost point on the colour profile) of the quadruplicate analysis of standard. In this analysis the pixel colour intensity peak showed an increase and the concentration increased. Since ammonia was detected using the Nessler reagent which resulted in intense yellow or brown colour, the red value gave the best sensitivity.

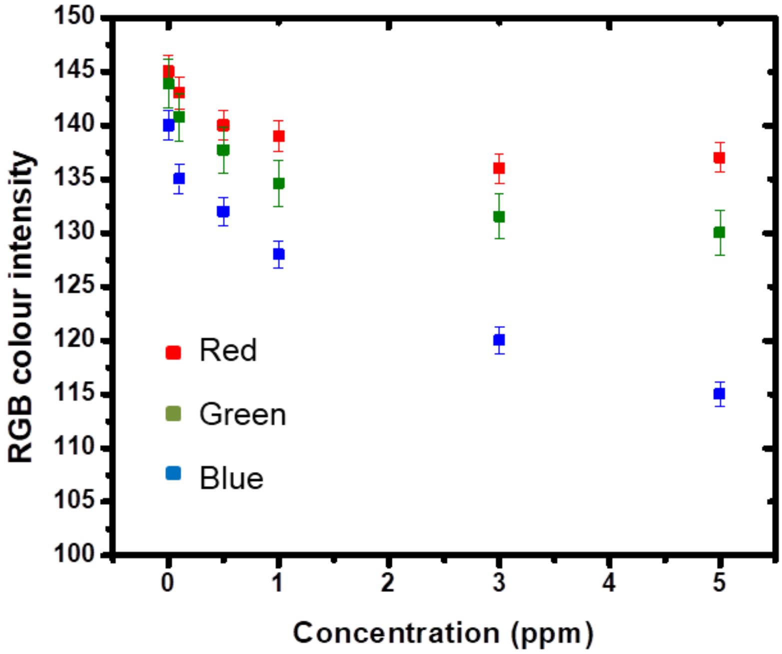


Figure 4.9 *RGB values for ammonia standards with the Nessler reagent*

The colour of the chromogenic reagent experiments showed a huge shift from pale yellow to brown or intense yellow when the ammonia concentration was increasing. It can be seen that there was a decrease in the intensity of the blue channel with the increased in ammonia concentration, while the intensity of red and green increased **(Fig 4.9)**. The RGB colour model is based on the science of the human eye preceives light and translates it into brain waves (Tkalcic and Tasic 2003; Menesatti *et al.* 2012; Alala, Mwangi and Okeyo 2014).

4.3.1.2 Salicylate method: Extraction of the RGB values

In the optimization of ammonia using the salicylate method, the final colour observed was green. The histograms for the total pixel values for red, green and blue are shown in **Annexure 2**.

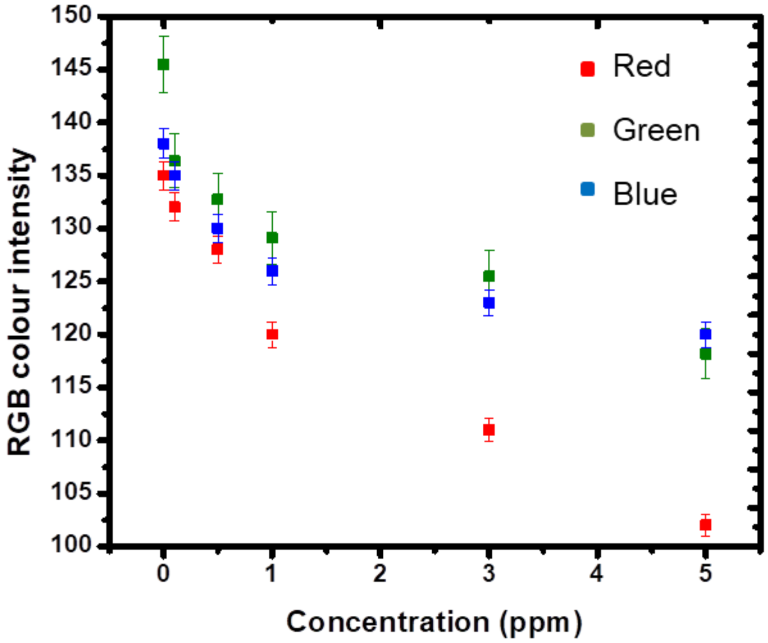


Figure 4.10 *RGB colour intensity extracted from digital images of the μPAD*

There was a decrease in the intensity of the red channel with the increase in ammonia concentration, while the intensity of blue and green increases observed in **Fig 4.10**. Thus, the final colour observed from this analysis was green, thus the RGB color model permits the colour that was visually observed by the naked eye.

The average of RGB values is one of the most common measure in image-analysis based colorimetry (Soldat, Barak and Lepore 2009; Shen, Hagen and Papautsky 2012). However, the RGB colour intensity does not allow quantitative analysis based on multiple changes of colour because the RGB cannot follow multiple colour changes as it can be seen in **Fig 4.6** and **Fig 4.7** (section 4.3.1 and 4.3.2). Thus, an effective method of quantifying colour is still needed for quantitative measurements and building calibration curves. Studies had revealed that the average of RGB values is one of the most common measure in image-analysis based colorimetry (Soldat, Barak and Lepore 2009; Shen, Hagen and Papautsky 2012). Thus, an effective method of quantifying colour is still needed for quantitative measurements and building calibration curves. Therefore, a CIELAB was further used for the accuracy of colour measurement. The CIELAB framework is a streamlined scientific estimate to a uniform colour space made out of apparent colour contrasts (Weatherall and Coombs 1992; Capitán-Vallvey *et al.* 2015; Romaniello, Leone and Peri 2015). The perceived lightness L* of a standard observer is assumed to follow the intensity of a colour magnification (Gonnet 1998; Capitán-Vallvey *et al.* 2015). The colours of lightness L* are arranged between the opponent colours green-red and blue-yellow along the rectangular coordinates a* and b*. The total difference between the two colours is given in terms of L*, a*, b* by the CIE 1976 formula (Hill, Roger and Vorhagen 1997; Mokrzycki and Tatol 2011).

4.3.2 CIELAB colour space

The uniform color space CIELAB, of the Commission Internationale de l'Eclairage, has been used by image processing researchers (Tominaga 1992; Kasson *et al.* 1995). CIEL*a*b* colour space is the first defined relationship between colours visualized by the human eye and the electromagnetic spectrum (McGuire 1992; Fairchild and Berns 1993). It was internationally adopted in the 1970's for the purpose of colour acceptability decision making from colour measurements by spectrophotometers and colorimeters. It was therefore designed for the inspection of flat objects, and has been used for this purpose to make acceptability decisions from color images, for color in fabrics, printed card and paper, and shade cards (McGuire 1992; Connolly, Leung and Nobbs 1995). Proper calibration and colour adjustment among the digital device is required for accurate colour measurements (Connolly and Fleiss 1997). In this study, the colour dependent device (average RGB values of the central region of the solution image) were converted to a standard device independent colour space which is CIEL*a*b*. The RGB values were converted to Lab using hunters lab (http://colormine.org/convert/rgb-to-hunterlab) inorder to obtained the CIE Lab (L-ligthness, a-yellow and -b- blue) colour space (Tkalcic and Tasic 2003). The main difference between the RGB and CIEL*a*b* colour spaces is implemented in the chromatic adaptation model. This color space includes all perceivable colours, it has a gamut that is greater than human vision.

The perceived lightness L* of a standard observer is assumed to follow the intensity of a colour magnification (Gonnet 1998; Capitán-Vallvey *et al.* 2015). The colours of lightness L* are arranged between the opponent colours green-red and blue-yellow along the rectangular coordinates a* and b*. The total difference between the two colours is given in terms of L*, a*, b* by the CIE 1976 formula (Hill, Roger and Vorhagen 1997; Mokrzycki and Tatol 2011). The CIE system (Tominaga 1992) is the most recognized method in which colour is represented by parameters x and y determining chromaticity of a colour and Y parameter representing luminance (brightness) of a colour. The colour space is then represented in a 2-D chromaticity diagram and can be used to predict outcome of colour mixtures (Hill, Roger and Vorhagen 1997). The chromaticity diagrams for the colorimetric detection of ammonia using the Nessler reagent method is displayed in **Annexure 3** and for the salicylate method **Annexure 4**. For the Nessler reagent method, it can be seen that as the concentration increases, the shift in the chromatic diagram is becoming more intense yellow whereas in the salicylate method it is becoming green. From the CIELAB the color difference was calculated. The obtained result for colour difference calculation of both the Nessler and salicylate method are shown in **Fig 4.11**.

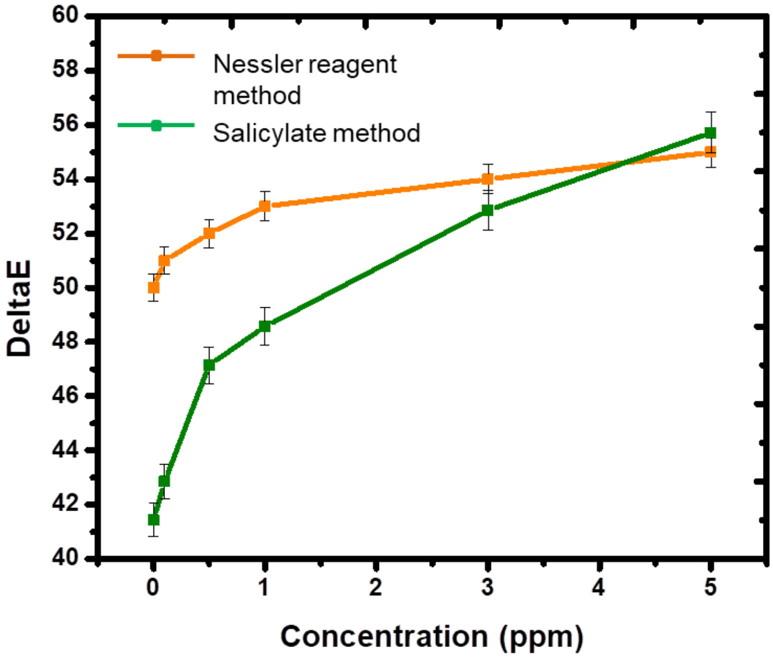


Figure 4.11 *ΔE observed in each concentration for both the salicylate and the Nessler reagent method*

It was noticed in **Fig 4.11** that the ΔE value increased monotonously with increasing standard concentration with small standard deviations. For the salicylate method, it can be seen that there is a sizeable colour difference observed between the standard, whereas for the Nessler reagent method, although there was a difference in the colour of the standard, but it was compact.

4.4 Analysis of real samples

4.4.1 Colorimetric approach

Wastewater samples which were collected from UIC ETP dam were colorimetrically analysed in order to detect and quantify ammonia. For the Nessler reagent method an intense yellow was observed and for the salicylate method, a green colour was observed, this is depicted in fig 4.12 below.

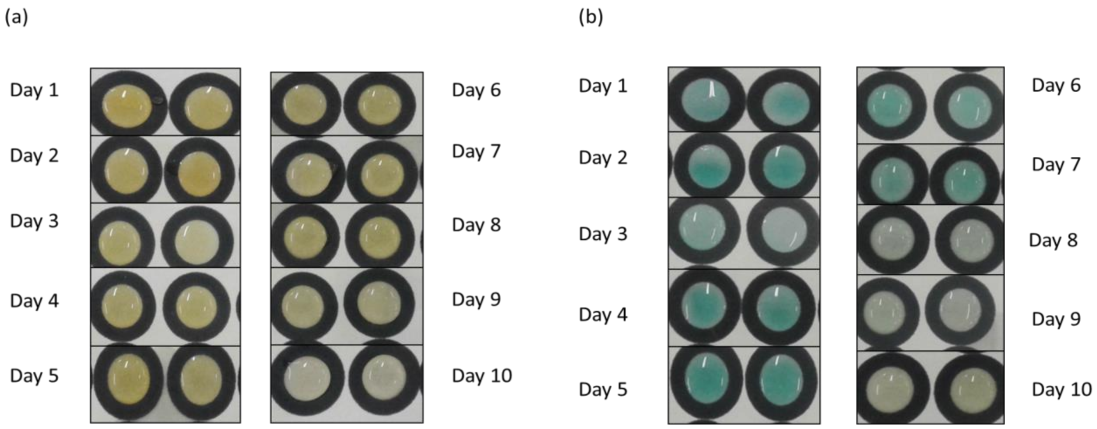


***Figure 4.12** colorimetric detection and quantification of ammonia using (a) Nessler reagent method and (b) Salicylate method*

As shown in **Fig 4.12** (a) and (b), both methods displayed that, varying amount of ammonia concentrations were detected from each sample. This was due to that, since these samples were collected during the working days of all the companies operation within the UIC, the load of their wastewater varied and the effect of temperature must have been encountered. Thus, as the loading rate increased, the effluent composition in the reactor changed to contain various combinations of ammonia. For the Nessler method, the change in colour intensity from pale yellow to intense yellow **Fig 4.12 (a)** suggested the presence of ammonia. This finding was further supported by the previous work of Jeong and co-workers that the presence of ammonia changes colour from pale yellow to intense yellow (Jeong, Park and Kim 2013). Particularily for the salicylate method the product colour was green as seen in **Fig 4.12 (b)** (Bower and Holm-Hansen 1980; Krom 1980). The concentration of ammonia taken in ten days was found to be dissimilar. The traces of ammonia detected in UIC wastewater samples were in ppm concentrations units (Table 4.4). From day 1, 2, 4, 5, 6, and 7, there was a visible colour change observed for both methods. The change in colour observed from these samples indicated the presence of ammonia on each method. The concentration of ammonia from day 1 to day 7 was found to be similar for both methods (see Table 4.4). Martinez-Carballo and co-workers (2007) suggested that the detected ammonia concentrations may be due to the matrix effects being higher in the influents than in the effluents of WWTPs which lead to higher matrix suppression and higher quantification limits in influents (Martínez-Carballo *et al.* 2007). In particular, naked eye observation of colour change from day 3, 8, 9, and 10, showed that the, there was a slight colour change, which was not visible enough. Therefore, ammonia concentration detected from these samples was low. The colorimetric analysis of both the Nessler reagent and the salicylate method indicated that the resulting ammonia concentrations obtained from the UIC ETP dam wastewaters samples was at the level of that of domestic wastewater which made it possible to be discharged directly to sewer as far as the nitrogen limitation was considered. However, it was observed that the salicylate procedure appeared to have better precision and could be used to detect small amount of ammonia present in sample solutions than the Nessler reagent method.

Table 4.4: Colorimetric results for the detection of ammonia in wastewater samples

ETP Dam samples	**Nessler method**		**Salicylate method**	
	Intensity	**Concentration (ppm)**	**Intensity**	**Concentration (ppm)**
Day 1	170	4.51	130	4.18
Day 2	165	3.85	123	3.16
Day 3	150	1.49	113	1.88
Day 4	158	2.70	122	3.01
Day 5	156	2.39	120	2.72
Day 6	155	2.24	119	2.58
Day 7	154	2.09	115	1.99
Day 8	143	0.43	105	0.54
Day 9	148	1.19	110	1.26
Day 10	158	0.10	102	0.10

The experimental data gathered confirmed that colorimetric detection of ammonia in wastewater using the µPAD was highly successful. The colorimetric approach results in Table 4.4 showed that the concentration obtained for both methods was essentially identical.

4.4.2 Spectrophotometric approach

The spectrophotometric results for the wastewater samples are shown in Table 4.5.

Table 4.5: Spectrophotometric results for the detection of ammonia in wastewater samples

ETP Dam samples	**Nessler method**		**Salicylate method**	
	Absorbance	**Concentration (ppm)**	**Absorbance**	**Concentration (ppm)**
Day 1	0.472	4.67	0.722	3.93
Day 2	0.345	3.32	0.623	3.35
Day 3	0.155	1.29	0.274	1.54
Day 4	0.241	2.55	0.552	2.94
Day 5	0.265	2.46	0.571	3.05
Day 6	0.213	1.91	0.425	2.21
Day 7	0.246	2.26	0.434	2.26
Day 8	0.088	0.57	0.1288	0.49
Day 9	0.158	1.32	0.2263	1.06
Day 10	0.045	0.11	0.0623	0.11

As noticed in Table 4.5, the spectrophotometric determination of ammonia was done using both the salicylate method at 630 nm and the Nessler method at 425 nm. Both the colorimetric and spectrophotometric approaches were used to evaluate the applicability of the developed µPAD for determination of ammonia in wastewater samples. It was observed that the results obtained from the spectrophotometric approach were similar to those of the colorimetric approach. Thus colorimetric method can serve as a rapid, simple and cheap alternative for the detection of ammonia in wastewater.

4.5 Statistical analysis of data

It has been stated that data analysis is fundamentally about data reduction and is concerned with lessening the substantial volume of information that the researcher has assembled so that he or she can understand of it (Bryman and Cramer 2012; Wahyuni 2012). Statistics has two broad genres namely, descriptive and inferential. In literature, it is elaborated that descriptive statistics describe, organise and summarise a particular set of quantitative data (Güler *et al.* 2002; Mason, Lind and Marchal 2002). Although such statistics makes no inference or predictions, they are useful in summarising results for an experiment. A bivariate descriptive statistical procedure was used to analyse the data in this study.

All results are depicted in mg L^{-1} (ppm) and the “n” number of the repetitive experiments completed shown alongside each experiment. The standards were quadruplicated and samples duplicated. The limit of detection or linearity values were compared using statistical models published in other paper microfluidic devices (Carrilho, Martinez and Whitesides 2009; Li, Tian and Shen 2010; Fu *et al.* 2011; Li, Ballerini and Shen 2012). A t-test was used to compare the statistical models used in the data (colorimetric and spectrophotometric data). A P value of < 0.05 was considered as significantly different, while that > 0.05 was not considered as significantly different.

In particular, the results obtained for the colorimetric detection of ammonia displayed (see **Fig 4.12** and Table 4.4) and those for spectrophotometric ammonia detection (Table 4.5) were statistically analysed to ensure there was no significant difference between the results from the paper device and results obtained from the UV Visible spectrophotometer. This was completed by using a paired student's t-test, which outlines there was no significant difference between the two approaches, (P= 0.26, Table 4.6). The results shown Table 4.6, indicates that the Salicylate method is more sensitive than the Nessler method. Nessler reagent (Wu and Cao 2013) have immense quantity of mercury salt, its toxicity and potential of secondary pollution is enormous. The salicylate spectrophotography method has a good interaction when the ammonia content is 0 - 5 mg L^{-1}.

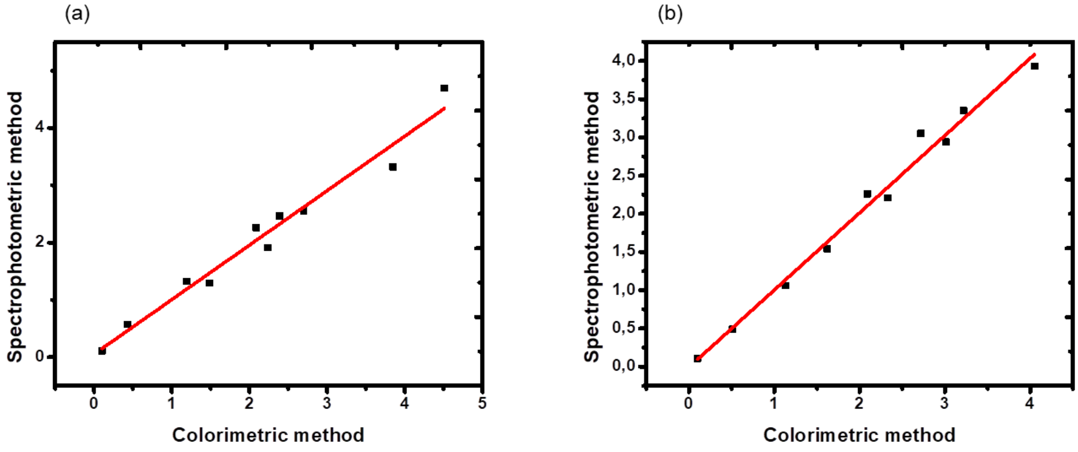


Figure 4.13 *Comparison of the spectrophotometric and colorimetric approach using (a) Nessler's reagent and (b) salicylate method*

The linear regression observed in **Fig 4.13** supports the paper device as an accurate method for the quantification of ammonia in wastewater samples via a high correlation between the paper device results and the colorimetric detection with Nessler reagent and salicylate method, R^2=0.9699 and R^2= 0.9857.

Table 4.6: t-Test: Paired Two Sample for Means

	Nessler's reagent method		**Salicylate method**	
	Variable 1	**Variable 2**	**Variable 1**	**Variable 2**
Mean	2,099	2,048	2,078	2,094
Variance	1,924832222	1,792084	1,55744	1,61158222
Observations	10	10	10	10
Pearson Correlation	0,984812001		0,99321538	
Hypothesized Mean Difference	0		0	
Df	9		9	
t Stat	0,665160811		-0,3414317	

P(T<=t) one-tail	0,261312889	0,37030746
t Critical one-tail	1,833112933	1,83311293
P(T<=t) two-tail	0,522625778	0,74061493
t Critical two-tail	2,262157163	2,26215716

Variable 1 colorimetric method, Variable 2 spectrophotometric method

Table 4.7: F-Test Two-Sample for Variances

	Nessler's reagent method		**Salicylate method**	
	Variable 1	**Variable 2**	**Variable 1**	**Variable 2**
Mean	2,099	2,048	2,094	2,078
Variance	1,924832	1,792084	1,6115822	1,55744
Observations	10	10	10	10
Df	9	9	9	9
F	1,074075		1,0347636	
P(F<=f) one-tail	0,458486		0,4801182	
F Critical one-tail	3,178893		3,1788931	

The linear regression observed in **Fig 4.13** supports the paper device as an accurate method for the quantification of ammonia in wastewater samples via a high correlation between the paper device results and the colorimetric detection with Nessler reagent and salicylate method, R^2=0.9699 and R^2= 0.9857.

4.6 Colour measurement for real samples

4.6.1 RGB colour intensity for Nessler reagent method

The colorimetric image of ammonia detected in wastewater using the Nessler reagent in section 4.5.1 **(Fig 4.12 (a))** was analysed for RGB values using Image J software. The obtained RGB colour histograms showing the pixels and the RGB values of each wastewater samples performed in duplicate analysis are depicted in **Annexure 5**. In a close look of the RGB pixels, it was observed that, for the more intense yellow coloured samples, their R and G values were shifting closer to the region of 225, and the B value coming closer to 0. The average RGB values of each duplicated samples was taken and used to plot the graph of RGB colour intensity against each wastewater sample as seen in **Fig 4.14**. It was observed that there was an increase in the R value, while the G and B values were decreasing.

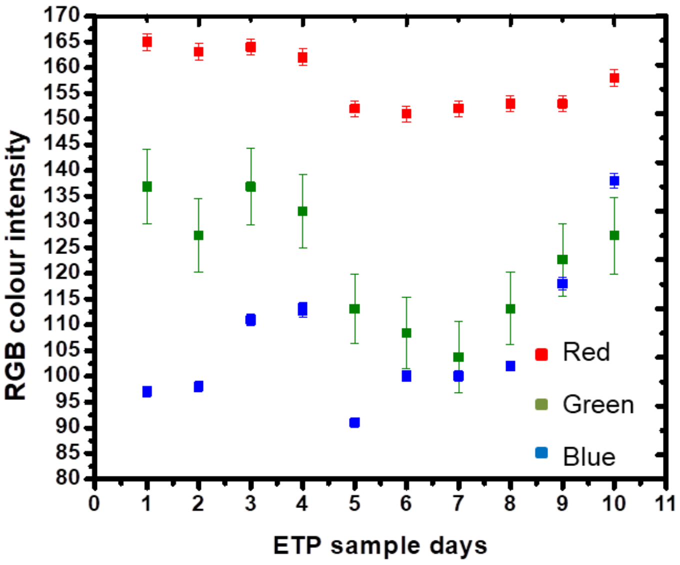


Figure 4.14 *RGB colour intensity for the colorimetric detection of ammonia in wastewater samples using Nessler reagent method*

4.6.2 RGB colour intensity of the salicylate method

The RGB colour intensity values of wastewater samples with the salicylate reagent were analysed using ImageJ software. The colorimetric image in section 4.5.1 **(Fig 4.12(b))** was analysed. The RGB values showing the pixel on each sample are shown in **Annexure 6**. The colour intensity of these values was measured by representing each pixel in the image with a numerical value between 0 and 255, where 0 is the esteem relegated to the darkest pixels and 255 is doled to the brilliant pixels.

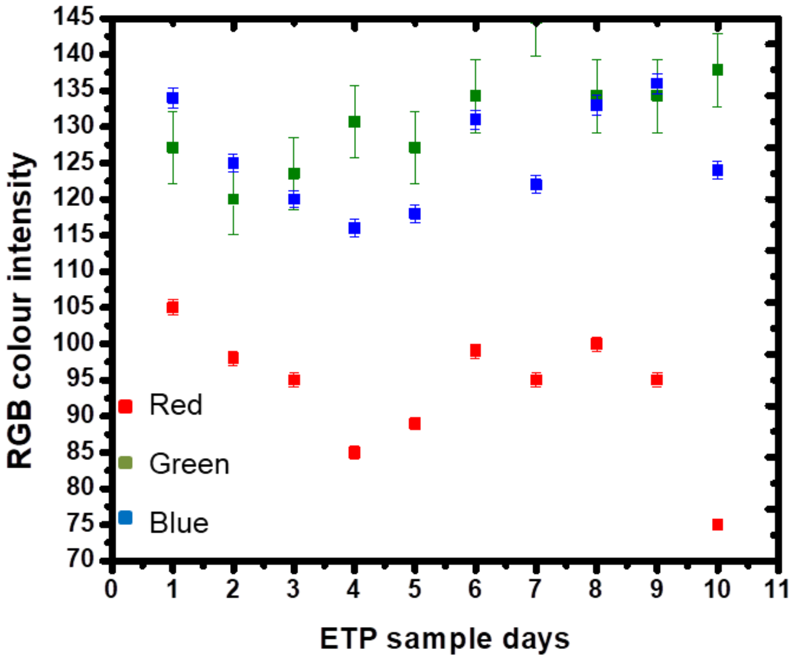


Figure 4.15 *RGB colour intensity for the colorimetric detection of ammonia in wastewater samples using salicylate method*

The R colour profile showed a shift to the left (lower pixel values) that reflected the darkening in colour due to the increase in green colour of the standard within the paper, whereas the G and B colour profiles shifted to the right, indicating an increase in colour. This was further supported by taking the average RGB values of each duplicated sample and plotting **Fig 4.15**. It was observed that, the RGB colour model detects the formation of the colour observed for the reaction of salicylate and the hypochlorite with ammonia in a similar principle as the naked eye could detect.

4.6.3 CIELAB colour space of real samples

The RGB colour model perhaps does not permit quantitative investigation dependent on numerous progressions of colour; for example, colorimetric test strips on the grounds that the RGB show cannot pursue various colour changes (Menesatti *et al.* 2012; Cai *et al.* 2014). Therefore a simple colorimetric method using a µPAD based on the CIE L*a*b* colour system was reported (Gonnet 1998). This model was carried out for both the colorimetric detection of ammonia in wastewater samples using Nessler reagent method and the salicylate method. The chromaticity diagrams of each duplicated sample for both methods are shown in **Annexure 7** and **Annexure 8**. The obtained L*a*b* values of the CIELAB colour space were used to calculate the colour difference on each colour obtained. It was observed that the colour of each sample was different from the other as shown in pattern of **Fig 4.16**.

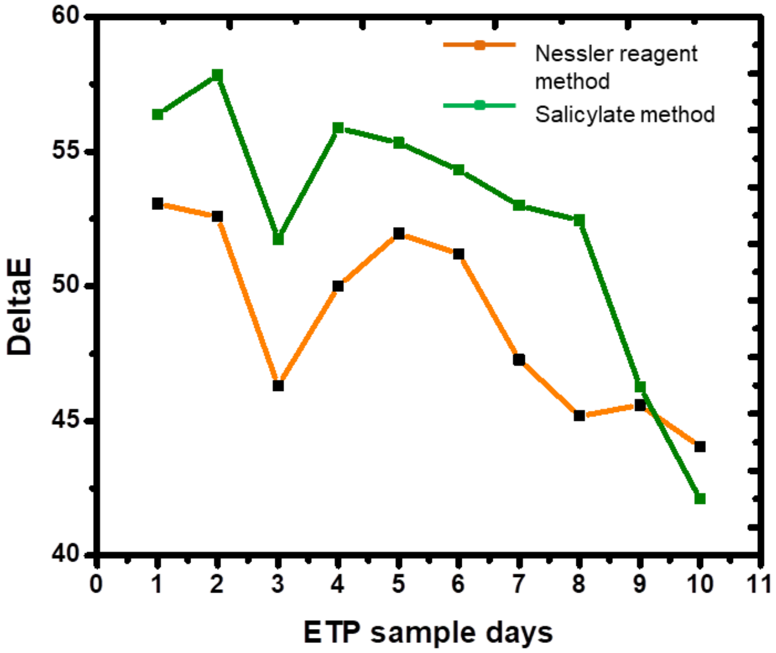


Figure 4.16 *Colour difference for colorimetric detection of ammonia using the Nessler reagent and the salicylate method*

4.7 The recoveries for the determination of ammonia in wastewater sample

Since it was observed that the salicylate method had better precision compared to the Nessler reagent method, recoveries of this method were used as measure of the accuracy of the methods. Samples containing spiked ammonia standard solutions were analysed and represented in Table 4.8. The recoveries of ammonia were in the range of 97.38 and 103.33%. These recoveries indicated that the proposed µPAD can be used for the determination of ammonia in wastewater samples.

Table 4.8: Recoveries of ammonia in wastewater (n=2)

Samples	**Concentration added**	**Concentration found**	**% recovery**	**RDS %**
1	3	3,10	103,33	1,78
2	3,1	3,11	100,19	1,84
3	3,2	3,12	97,38	1,89
4	3,3	3,25	98,48	1,87
5	3,4	3,41	100,29	1,84
6	3,5	3,49	99,57	1,85
7	3,6	3,55	98,61	1,87
8	3,7	3,71	100,27	1,84
9	3,9	4,01	102,82	1,79
10	4	4,02	100,50	1,83

4.8 The effect of interfering ions on ammonia determination

It is imperative to study the interferences to guarantee the development of the µPAD can accomplish effective analytical results in different sample matrices. The presence of iron (Fe^{2+}) copper (Cu^{2+}), calcium (Ca^{2+}), nickel (Ni^{2+}) and magnesium (Mg^{2+}) can interfere with the process of determining the ammonia content (Verdouw, Van Echteld and Dekkers 1978; Suneetha and Ravindhranath 2012). The effect of potential interferences upon the detection of ammonia in wastewater was studied by spiking each interference ion into ammonia sample. The observed colour change image is depicted in **Fig. 4.17**. Quantitative recoveries of the analyte are shown in Table 4.9.

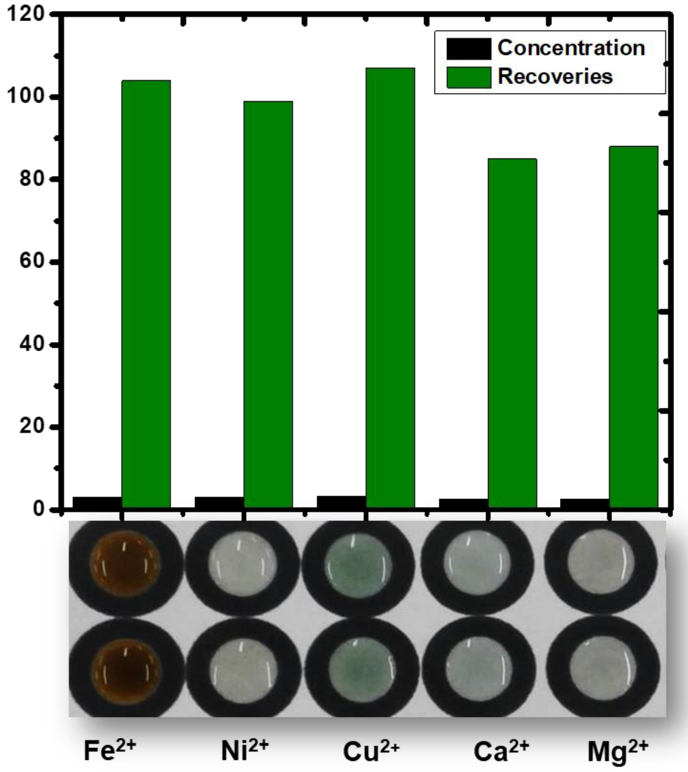


Figure 4.17 *Colorimetric detection of ammonia interfering ions in wastewater*

It was observed that for Fe^{2+} ammonia interfering ion, there was no green colour, Instead, a yellow-brown colour with gelatinous precipitate was observed. For the Cu^{2+} ion, a green colour indicating the presence of ammonia with a concentration of 3.22 mg L^{-1}. A very pale green colour was observed for Ni^{2+}, Ca^{2+}, and Mg^{2+} ions.

These interferences showed that they do interfere with the detection of ammonia; however, they have no significant impacts.

Table 4.9: Effect of interfering ions on ammonia determination in wastewater (n=2)

Interferences	**concentration (ppm)**	**% recovery**
Fe^{2+}	3.11	104
Cu^{2+}	3.22	107
Mg^{2+}	2.65	88
Ni^{2+}	2.98	99
Ca^{2+}	2.55	85

4.9 The optimization of Lateral flow microfluidic device

In this study the lateral flow analysis of ammonia was done using both the Nessler reagent and the salicylate method. The standards of ammonia were dropped into each reservoir, allowing the standards for quadruplicated analysis. The reagents were loaded on the center of the µPAD and were then allowed to spread to each reaction reservoir where it is neutralized by different amounts of the primary standard substance. The distance and time of each reagent flow through the capillary walls/passage in to the reservoir of each standard was measured. Colorimetric detection of ammonia for the lateral flow analysis is depicted in figure 5.1 below.

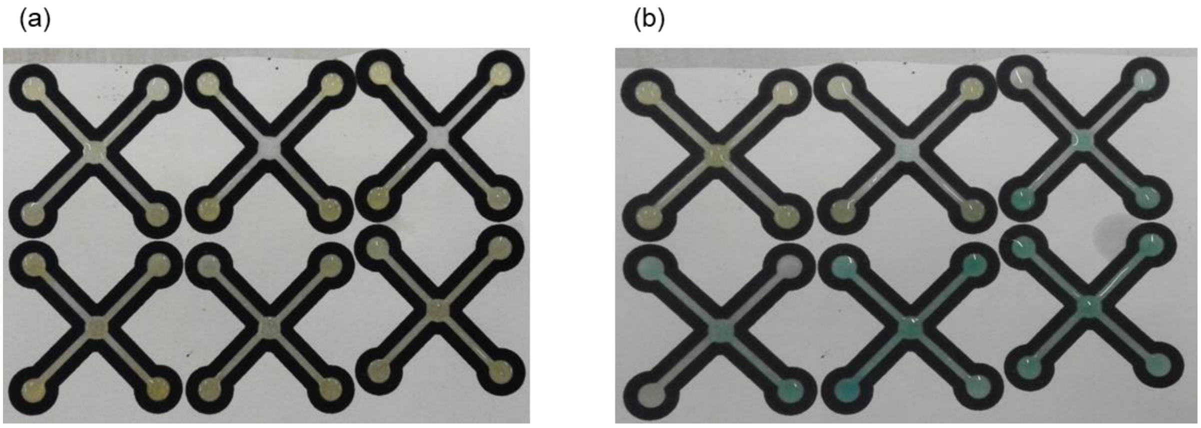


***Figure 4.18** Lateral flow assay of ammonia standards (0-5 mgL^{-1}) for (a) Nessler reagent method and (b) Salicylate method*

The colorimetric lateral flow analysis of ammonia using both the Nessler reagent and the salicylate method in **Fig 5.1** showed a final colour of brown-yellow and green, respectively. It was observed that when the reagents are loaded to the center of the μPAD, they move to the quadruplicate reservoirs of each standard at a certain distance and time. Although the time for the reagent flow in both methods varied, the measured flow distance to which the reagent transports from the center of the μPAD to the reservoirs of the standards was the same for both methods (Table 5.1). The elapsed time prior to analysis colour readout was considered important in paper-based microfluidic device design for the selection of the correct quantity of reagents for a highly sensitive and selective detection reaction. Another crucial concern, the design of a paper-based microfluidic device liaises to the user the ideal colour change readout time for the analysis to provide the most accurate results. The wicking time for the reagents to reach the full length on the reservoirs had a consistent value of approximately 0-90 seconds for Nessler reagent method and 5-120 seconds for the salicylate method. The distance at which the reagent fills the full length of the reservoir was observed to be approximately 5-55 mm for both methods.

Table 4.10: Experimental results for the lateral flow analysis of ammonia

Concentration (ppm)		**Nessler reagent method**	**Salicylate method**
	Distance (mm)	**Time (s)**	**Time (s)**
0	5	0	5
0.1	15	5	15
0.5	25	15	30
1	35	35	50
3	45	60	80
5	55	90	120

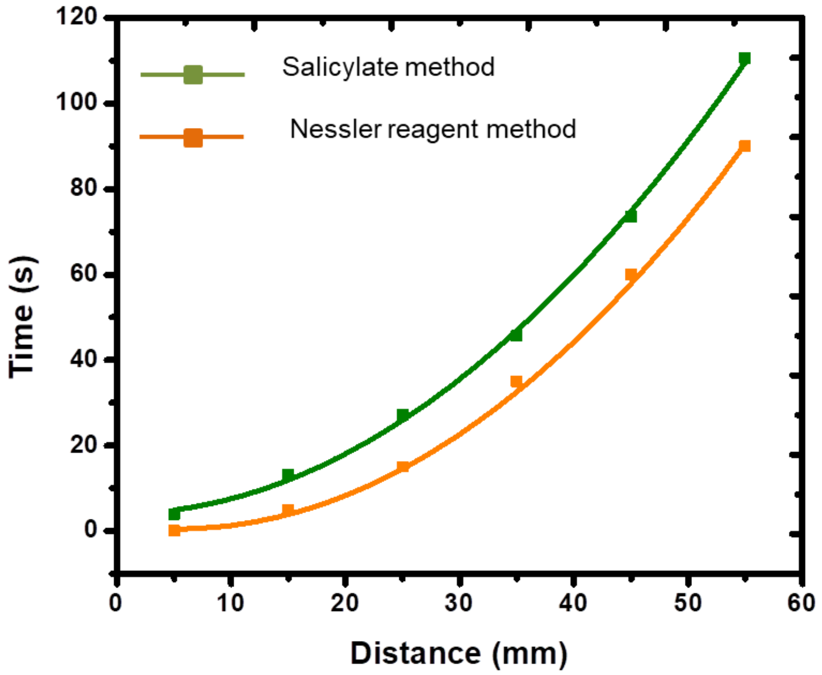


Figure 4.19 *Experimental observation of the lateral flow analysis of ammonia for both the Nessler reagent and the salicylate methods*

The results presented in **Fig 5.2**, clearly showed that the flow characteristic for both methods, a minimal perceptual colour difference between standards was observed. The interactions of flowing analyte in paper with reagents applied to the paper prior to wicking can be further investigated. It is imperative to understand the motion during imbibition of reagents that are placed on the paper. The closed channel flow system offers several benefits including increased flow rates, lower liquid retention, lower sample evaporation and a smaller sample volume requirement when compared to traditional filter-paper based µPADs.

CHAPTER FIVE

REVIEW, CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion and recommendations

5.1.1 Conclusion

Wastewater, produced from human activities has many negative effects on the off chance that it is insufficiently treated. Satisfactory treatment requires different procedures to guarantee consistence with release necessities and no antagonistic impacts on the water nature of the receiving water system. Given the lethal nature of ammonia segments found in the ETP of many industries, the wastewater containing this constituent can possibly affect adversely on both the environment and on human wellbeing, if mistakenly oversaw. Of immediate importance for the boundless appropriation of µPADs in routine water investigation is further enhancement in their affectability to make them a feasible and appealing option in contrast to customary and settled diagnostic bunch and flow analysis techniques for field applications.

Throughout the years, µPADs emerged as a promising platform for the development of expedite and inexpensive analytical solutions in developing and developed nations. In this work, a simple, cost effective and novel method for the fabrication of µPAD by chemical patterning of a chromatographic paper is reported. The method is exceedingly pertinent for point-of care testing particularly in asset restricted environments. Particularly, the current investigation has exhibited the likelihood of utilizing the prevalent wax-printing innovation joined with the most broadly utilized filter paper substrate to get scaled down paper-based analytical device with microfluidic structures, empowering the examination of tests just accessible in petite volumes. It has been exhibited that cell phones and tablets with particular portable applications are exceptionally valuable not just in the catch of a µPAD's picture (i.e. perception), yet additionally progressively preparing of this picture with the likelihood of ensuing sparing and transmission of the explanatory information. Such apparatuses reinforce essentially the movability and ease of use of µPADs. The designed µPAD was used for colorimetric detection of ammonia in wastewater. The principle capacity of this paper was to report the optimisation parameters of a clarified salicylate and Nessler reagent technique to encourage the coordination of the methods into an investigation framework, bringing about a straightforward methodology for the constant estimation of ammonia.

The results for the colorimetric approach presented analytical figures merits such as the low limit of detection of 3.37 mg L^{-1} achieved for the Nessler reagent method and 3.20 mg L^{-1} for the salicylate method On the other hand, the validation of both methods by the spectrophotometric approach gave a limit of detection of 0.52 mg L^{-1} for the Nessler reagent method and 0.75 mg L^{-1} for the salicylate method. The excellent correlation was also achieved with the reference (spectrophotometric) method. Therefore, the results for this study suggest that the salicylate and the Nessler reagent method can both be used across a wide range for colorimetric analysis of ammonia in wastewater. However, the use of mercury for the Nessler reagent method in this test warrants its omission on account of it ejection issues (Association *et al.* 1915; Rice *et al.* 2012), it is thus, not adequate for the determination of ammonia in wastewater whereas, the salicylate spectrophotography method has a good linear interaction when the ammonia content is 0-5 mg L^{-1}. Both the colorimetric and spectrophotometric approaches prove that, the salicylate method is more precise than the Nessler method. Thus, the successful application of the µPAD in the ammonia detection in wastewater demonstrates its potential for environmental monitoring. Furthermore, a simple ammonia in wastewater measurement in the µPAD by a colorimetric method dependent on the RGB model and CIE L*a*b* colour difference (ΔE) was illustrated. The use of ΔE was suitable for a colorimetric ammonia detection based on the multiple colour changes. The combination of the µPAD and image analysis based on colour difference ΔE enabled quantitative analysis for multiple colour changes (Capitán-Vallvey *et al.* 2015). The present paper-based scientific strategy will quicken applications to purpose of consideration testing and in asset restricted settings. Colorimetric detection provides a suitable, mobile, and quick approach to measure exposure at the time of need, though spectrophotometric strategy requires more costly and protracted, offsite investigations. In addition, the desirable recoveries demonstrate the reliability of the proposed method for the detection of ammonia in practical applications. In summary, this chapter outlined the characteristics of ammonia in terms of solubility, colour and alkalinity. These factors are fundamental in the development of µPADs

5.1.2 Recommendations

Future improvements will concentrate at first on enhancing the goals of the framework for the quantification of low ammonia concentrations. Subsequently, the significant accentuation is on field arrangements with this adjusted methodology for environmental monitoring. The need will be on the main problems associated with the investigative methodology and sampling within environmental waters/wastewaters and specifically, achieving free operation of the µPAD platform over extended periods of time. Furthermore, work could be done to exploit the chromatographic ability of paper. Considering the continuous nature of wastewater treatment systems, performance of the µPAD for colorimetric determination of ammonia in continuous mode needs to be studied.

The study focused on assessing the presence of ammonia on the effluent treatment plant of the uMbogintwini industrial complex in KwaZulu-Natal. Future studies could investigate the presence of ammonia in other business sectors in KwaZulu-Natal; for the entire province of KwaZulu-Natal, and/or in other provinces in South Africa

The SEM micrographs in this thesis demonstrated the complex structure of the paper fibre network. Impacts of fibre properties, such as versatility and swelling, should be investigated to determine their impact on analyte flow through paper media.

The µPAD serves as an alternative detection platform to current colorimetric methods and as a faster analysis approach for measuring a set volume solution in laboratory analysis for lateral flow assay. Therefore, several parameters involved in the development of the µPAD for lateral flow analysis can be studied and optimized including wax melting temperature, the optimum barrier width, the channel width etc.

REFERENCES

Abe, K., Suzuki, K. and Citterio, D. 2008. Inkjet-Printed Microfluidic Multianalyte Chemical Sensing Paper. *Analytical Chemistry*, 80 (18): 6928-6934.

Adler, R. A., Claassen, M., Godfrey, L. and Turton, A. R. 2007. Water, mining, and waste: an historical and economic perspective on conflict management in South Africa. *The economics of peace and security journal*, 2 (2)

Akpor, O. 2011. Wastewater effluent discharge: Effects and treatment processes. In: Proceedings of *Int. Conf. Chem. Biol. Environ. Eng*. 85-91.

Alala, B., Mwangi, W. and Okeyo, G. 2014. Image representation using RGB color space. *International Journal of Innovative Research and Development*, 3 (8)

Almeida, M. I. G. S., Estela, J. M., Segundo, M. A. and Cerdà, V. 2011. A membraneless gas-diffusion unit – multisyringe flow injection spectrophotometric method for ammonium determination in untreated environmental samples. *Talanta*, 84 (5): 1244-1252.

Amaratunga, D., Baldry, D., Sarshar, M. and Newton, R. 2002. Quantitative and qualitative research in the built environment: application of “mixed” research approach. *Work study*, 51 (1): 17-31.

Amirjani, A. and Fatmehsari, D. H. 2018. Colorimetric detection of ammonia using smartphones based on localized surface plasmon resonance of silver nanoparticles. *Talanta*, 176: 242-246.

Ansari, A. A., Gill, S. S. and Khan, F. A. 2010. Eutrophication: threat to aquatic ecosystems. In: *Eutrophication: causes, consequences and control*. Springer, 143-170.

Association, A. P. H., Association, A. W. W., Federation, W. P. C. and Federation, W. E. 1915. *Standard methods for the examination of water and wastewater*. American Public Health Association.

Ballerini, D. R., Li, X. and Shen, W. 2012. Patterned paper and alternative materials as substrates for low-cost microfluidic diagnostics. *Microfluidics and Nanofluidics*, 13 (5): 769-787.

Bhakta, S. A., Borba, R., Taba, M., Garcia, C. D. and Carrilho, E. 2014. Determination of nitrite in saliva using microfluidic paper-based analytical devices. *Analytica Chimica Acta*, 809: 117-122.

Blankenstein, G. 2000. Microfluidic devices for biomedical applications. *MST NEWS*: 14-15.

Bouman, O., Curtin, D., Campbell, C., Biederbeck, V. and Ukrainetz, H. 1995. Soil acidification from long-term use of anhydrous ammonia and urea. *Soil Science Society of America Journal*, 59 (5): 1488-1494.

Bourgeois, W., Burgess, J. E. and Stuetz, R. M. 2001. On-line monitoring of wastewater quality: a review. *Journal of chemical technology and biotechnology*, 76 (4): 337-348.

Bouwer, H. 2002. Integrated water management for the 21st century: problems and solutions. *Journal of Irrigation and Drainage Engineering*, 128 (4): 193-202.

Bouwer, H. and Idelovitch, E. 1987. Quality requirements for irrigation with sewage water. *Journal of irrigation and drainage engineering*, 113 (4): 516-535.

Bower, C. E. and Holm-Hansen, T. 1980. A salicylate–hypochlorite method for determining ammonia in seawater. *Canadian Journal of Fisheries and Aquatic Sciences*, 37 (5): 794-798.

Brautbar, N., Wu, M. P. and Richter, E. D. 2003. Chronic ammonia inhalation and interstitial pulmonary fibrosis: a case report and review of the literature. *Archives of Environmental Health: An International Journal*, 58 (9): 592-596.

Brooke, L. and Thursby, G. 2005. Aquatic life ambient water quality criteria—nonylphenol. *US Environmental Protection Agency (EPA), Office of Water Office of Science and Technology, Washington DC*, 88

Bruzewicz, D. A., Reches, M. and Whitesides, G. M. 2008. Low-Cost Printing of Poly(dimethylsiloxane) Barriers To Define Microchannels in Paper. *Analytical Chemistry*, 80 (9): 3387-3392.

Bryman, A. and Cramer, D. 2012. *Quantitative data analysis with IBM SPSS 17, 18 & 19: A guide for social scientists*. Routledge.

Busa, L. S. A., Mohammadi, S., Maeki, M., Ishida, A., Tani, H. and Tokeshi, M. 2016. Advances in microfluidic paper-based analytical devices for food and water analysis. *Micromachines*, 7 (5): 86.

Cai, L., Wang, Y., Wu, Y., Xu, C., Zhong, M., Lai, H. and Huang, J. 2014. Fabrication of a microfluidic paper-based analytical device by silanization of filter cellulose using a paper mask for glucose assay. *Analyst*, 139 (18): 4593-4598.

Caicedo, H. H. and Brady, S. T. 2016. Microfluidics: the challenge is to bridge the gap instead of looking for a ‘killer app’. *Trends in biotechnology*, 34 (1): 1-3.

Camplisson, C. K., Schilling, K. M., Pedrotti, W. L., Stone, H. A. and Martinez, A. W. 2015. Two-ply channels for faster wicking in paper-based microfluidic devices. *Lab on a Chip*, 15 (23): 4461-4466.

Capitán-Vallvey, L. F., Lopez-Ruiz, N., Martinez-Olmos, A., Erenas, M. M. and Palma, A. J. 2015. Recent developments in computer vision-based analytical chemistry: A tutorial review. *Analytica Chimica Acta*, 899: 23-56.

Cardoso, T. M. G., de Souza, F. R., Garcia, P. T., Rabelo, D., Henry, C. S. and Coltro, W. K. T. 2017. Versatile fabrication of paper-based microfluidic devices with high chemical resistance using scholar glue and magnetic masks. *Analytica Chimica Acta*, 974: 63-68.

Carrilho, E., Martinez, A. W. and Whitesides, G. M. 2009. Understanding wax printing: a simple micropatterning process for paper-based microfluidics. *Anal Chem*, 81 (16): 7091-7095.

Carvalhal, R. F., Carrilho, E. and Kubota, L. T. 2010. The potential and application of microfluidic paper-based separation devices. *Bioanalysis*, 2 (10): 1663-1665.

Cate, D. M., Adkins, J. A., Mettakoonpitak, J. and Henry, C. S. 2014. Recent developments in paper-based microfluidic devices. *Analytical Chemistry*, 87 (1): 19-41.

Cate, D. M., Dungchai, W., Cunningham, J. C., Volckens, J. and Henry, C. S. 2013. Simple, distance-based measurement for paper analytical devices. *Lab on a Chip*, 13 (12): 2397-2404.

Chapman, D. V. and Organization, W. H. 1996. Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring.

Chruszcz-Lipska, K., Knapik, E., Rychlicki, S. a. and Stopa, J. 2015. Assessment of the quality of surface water from selected area of active oil exploitation. *AGH Drilling, Oil, Gas*, 32: 65-76.

Close, L. G., Catlin, F. I. and Cohn, A. M. 1980. Acute and chronic effects of ammonia burns of the respiratory tract. *Archives of Otolaryngology*, 106 (3): 151-158.

Cogan, D., Cleary, J., Fay, C., Rickard, A., Jankowski, K., Phelan, T., Bowkett, M. and Diamond, D. 2014. The development of an autonomous sensing platform for the monitoring of ammonia in water using a simplified Berthelot method. *Analytical Methods*, 6 (19): 7606-7614.

Colt, J. and Huguenin, J. E. 2002. *Design and operating guide for aquaculture seawater systems*. Elsevier.

Comer, J. P. 1956. Semiquantitative Specific Test Paper for Glucose in Urine. *Analytical Chemistry*, 28 (11): 1748-1750.

Connolly, C. and Fleiss, T. 1997. A study of efficiency and accuracy in the transformation from RGB to CIELAB color space. *IEEE Transactions on Image Processing*, 6 (7): 1046-1048.

Connolly, C., Leung, W. and Nobbs, J. 1995. Colour measurement by video camera. *Journal of the Society of Dyers and Colourists*, 111 (12): 373-375.

Constable, M., Charlton, M., Jensen, F., McDonald, K., Craig, G. and Taylor, K. W. 2003. An ecological risk assessment of ammonia in the aquatic environment. *Human and Ecological Risk Assessment*, 9 (2): 527-548.

Credou, J. and Berthelot, T. 2014. Cellulose: from biocompatible to bioactive material. *Journal of Materials Chemistry B*, 2 (30): 4767-4788.

Dalton, H. and Brand-Hardy, R. 2003. Nitrogen: the essential public enemy. *Journal of Applied Ecology*, 40 (5): 771-781.

De Bode, M. 1991. Odour and ammonia emissions from manure storage. *Odour and ammonia emissions from livestock farming*, 5: 59.

Demutskaya, L. N. and Kalinichenko, I. E. 2010. Photometric determination of ammonium nitrogen with the nessler reagent in drinking water after its chlorination. *Journal of Water Chemistry and Technology*, 32 (2): 90-94.

Diersing, N. and Nancy, F. 2009. Water quality: Frequently asked questions. *Florida Brooks National Marine Sanctuary, Key West, FL*,

Dobler, L. J., Gibbons, J. M. and Evtodienko, V. Y. 2006. *Quick acting toxic ammonia test for aqueous samples*: Google Patents.

Dou, M., Sanjay, S. T., Benhabib, M., Xu, F. and Li, X. 2015. Low-cost bioanalysis on paper-based and its hybrid microfluidic platforms. *Talanta*, 145: 43-54.

Dungchai, W., Chailapakul, O. and Henry, C. S. 2009. Electrochemical detection for paper-based microfluidics. *Analytical Chemistry*, 81 (14): 5821-5826.

Dungchai, W., Chailapakul, O. and Henry, C. S. 2011. A low-cost, simple, and rapid fabrication method for paper-based microfluidics using wax screen-printing. *Analyst*, 136 (1): 77-82.

Duong, H. D. and Rhee, J. I. 2014. A ratiometric fluorescence sensor for the detection of ammonia in water. *Sensors and Actuators B: Chemical*, 190: 768-774.

Duong, H. H. 2016. Development of Simultaneous Measurement Method of Gaseous Ammonia and Fine Particulate Ammonium and Study on Their Behaviors in Urban Atmosphere.

Ellerbee, A. K., Phillips, S. T., Siegel, A. C., Mirica, K. A., Martinez, A. W., Striehl, P., Jain, N., Prentiss, M. and Whitesides, G. M. 2009. Quantifying Colorimetric Assays in Paper-Based Microfluidic Devices by Measuring the Transmission of Light through Paper. *Analytical Chemistry*, 81 (20): 8447-8452.

Erguder, T. H., Boon, N., Wittebolle, L., Marzorati, M. and Verstraete, W. 2009. Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. *FEMS microbiology reviews*, 33 (5): 855-869.

Erisman, J. and Schaap, M. 2004. The need for ammonia abatement with respect to secondary PM reductions in Europe. *Environmental Pollution*, 129 (1): 159-163.

Erisman, J. W., Bleeker, A., Galloway, J. and Sutton, M. S. 2007. Reduced nitrogen in ecology and the environment. *Environmental Pollution*, 150 (1): 140-149.

Fairchild, M. D. and Berns, R. S. 1993. Image color-appearance specification through extension of CIELAB. *Color Research & Application*, 18 (3): 178-190.

Fedoruk, M. J., Bronstein, R. and Kerger, B. D. 2005. Ammonia exposure and hazard assessment for selected household cleaning product uses. *Journal Of Exposure Analysis And Environmental Epidemiology*, 15: 534.

Ferreira, A. M., Lima, J. and Rangel, A. 1996. Potentiometric determination of total nitrogen in soils by flow injection analysis with a gas-diffusion unit. *Soil Research*, 34 (4): 503-510.

Fu, E., Ramsey, S. A., Kauffman, P., Lutz, B. and Yager, P. 2011. Transport in two-dimensional paper networks. *Microfluidics and nanofluidics*, 10 (1): 29-35.

Galvão, J. A., Matthiensen, A., Oetterer, M., Moliner-Martínez, Y., Gonzalez-Fuenzalida, R., Muñoz-Ortuño, M., Herráez-Hernández, R., Verdú-Andrés, J., Molins-Legua, C. and Falcó, P. C. 2013. *Determination of ammonia in water samples*: CRC Press: Boca Raton.

George, A., Bang, R. L., Lari, A.-R., Gang, R. K. and Kanjoor, J. R. 2000. Liquid ammonia injury. *Burns*, 26 (4): 409-413.

Gerber, P., Opio, C. and Steinfeld, H. 2007. Poultry production and the environment—a review. *Animal Production and Health Division, Food and Agriculture Organization of the United Nations, Viale delle Terme di Caracalla. Room, Italy*, 153

Gonnet, J.-F. 1998. Colour effects of co-pigmentation of anthocyanins revisited—1. A colorimetric definition using the CIELAB scale. *Food Chemistry*, 63 (3): 409-415.

Govindasamy, K., Potgieter, S., Land, K. and Muzenda, E. 2012. Fabrication of paper based microfluidic devices. 978-988.

Güler, C., Thyne, G. D., McCray, J. E. and Turner, K. A. 2002. Evaluation of graphical and multivariate statistical methods for classification of water chemistry data. *Hydrogeology journal*, 10 (4): 455-474.

Haber, F. 2002. The synthesis of ammonia from its elements Nobel Lecture, June 2, 1920. *Resonance*, 7 (9): 86-94.

Halling-Sørensen, B. and Jorgensen, S. E. 1993. *The removal of nitrogen compounds from wastewater*. Elsevier.

Hart, R. C. and Allanson, B. R. 1984. *Limnological criteria for management of water quality in the southern hemisphere*. National Scientific Programmes Unit: CSIR.

Hemson, D. 2016. Water, sanitation and health: South Africa's remaining and existing issues. *South African Health Review*, 2016 (1): 25-34.

Hill, B., Roger, T. and Vorhagen, F. W. 1997. Comparative analysis of the quantization of color spaces on the basis of the CIELAB color-difference formula. *ACM Transactions on Graphics (TOG)*, 16 (2): 109-154.

Ho, Y., Show, K., Guo, X., Norli, I., Abbas, F. A. and Morad, N. 2012. Industrial discharge and their effect to the environment. In: *Industrial Waste*. InTech.

Hodgson, K. and Manus, L. 2006. A drinking water quality framework for South Africa. *Water SA*, 32 (5)

Hopkins, W. G. 2008. Quantitative research design.

Hu, J., Wang, S., Wang, L., Li, F., Pingguan-Murphy, B., Lu, T. J. and Xu, F. 2014. Advances in paper-based point-of-care diagnostics. *Biosensors and Bioelectronics*, 54: 585-597.

Hunt, R. W. G. and Pointer, M. R. 2011. *Measuring colour*. John Wiley & Sons.

Hutton, W. and LaRocca, S. 1975. Biological treatment of concentrated ammonia wastewaters. *Journal (Water Pollution Control Federation)*: 989-997.

Jafarpour, M., Foolad, A., Mansouri, M., Nikbakhsh, Z. and Saeedizade, H. 2010. Ammonia removal from nitrogenous industrial waste water using iranan natural zeolite of clinoptilolite type. *World Academy of Science, Engineering and Technology*, 70 (2010): 939-945.

Jagadeesan, K. K., Kumar, S. and Sumana, G. 2012. Application of conducting paper for selective detection of troponin. *Electrochemistry Communications*, 20: 71-74.

Jayawardane, B. M., McKelvie, I. D. and Kolev, S. D. 2015. Development of a Gas-Diffusion Microfluidic Paper-Based Analytical Device (μPAD) for the Determination of Ammonia in Wastewater Samples. *Analytical Chemistry*, 87 (9): 4621-4626.

Jeong, H., Park, J. and Kim, H. 2013. Determination of NH4+ in Environmental Water with Interfering Substances Using the Modified Nessler Method. *Journal of Chemistry*, 2013: 9.

Jorgensen, S. E. 1975. Recovery of ammonia from industrial waste water. *Water Research*, 9 (12): 1187-1191.

Jorgensen, T. C. 2002. Removal of ammonia from wastewater by ion exchange in the presence of organic compounds.

Karri, R. R., Sahu, J. N. and Chimmiri, V. 2018. Critical review of abatement of ammonia from wastewater. *Journal of Molecular Liquids*, 261: 21-31.

Kasson, J. M., Nin, S. I., Plouffe, W. and Hafner, J. L. 1995. Performing color space conversions with three-dimensional linear interpolation. *Journal of Electronic Imaging*, 4 (3): 226-251.

Kerger, B. D. and Fedoruk, M. J. 2015. Pathology, toxicology, and latency of irritant gases known to cause bronchiolitis obliterans disease: Does diacetyl fit the pattern? *Toxicology Reports*, 2: 1463-1472.

Khopkar, S. 2007. *Environmental pollution monitoring and control*. New Age International.

Klimont, Z. 2001. Current and future emissions of ammonia in China. In: Proceedings of *10th annual emission inventory conference: one atmosphere, One inventory, many challenges. May*. 1-3.

Krom, M. D. 1980. Spectrophotometric determination of ammonia: a study of a modified Berthelot reaction using salicylate and dichloroisocyanurate. *Analyst*, 105 (1249): 305-316.

Kruse, J. and Mellon, M. 1953. Colorimetric determination of ammonia and cyanate. *Analytical Chemistry*, 25 (8): 1188-1192.

Kuo, W. and Zhu, X. 2012. *Importance measures in reliability, risk, and optimization: principles and applications*. John Wiley & Sons.

Larsen, B. K., Dalsgaard, J. and Pedersen, P. B. 2015. An optimized and simplified method for analysing urea and ammonia in freshwater aquaculture systems. *Aquaculture Research*, 46 (7): 1608-1618.

Lefebvre, O. and Moletta, R. 2006. Treatment of organic pollution in industrial saline wastewater: A literature review. *Water Research*, 40 (20): 3671-3682.

Li-qiong, C. and Yong, H. 2011. Influencing factors on determination of ammonia nitrogen in water by nessler's reagent spectrophotometry. In: Proceedings of *Water Resource and Environmental Protection (ISWREP), 2011 International Symposium on*. IEEE, 1173-1176.

Li, X., Ballerini, D. R. and Shen, W. 2012. A perspective on paper-based microfluidics: current status and future trends. *Biomicrofluidics*, 6 (1): 011301.

Li, X., Tian, J., Garnier, G. and Shen, W. 2010. Fabrication of paper-based microfluidic sensors by printing. *Colloids and Surfaces B: Biointerfaces*, 76 (2): 564-570.

Li, X., Tian, J., Nguyen, T. and Shen, W. 2008. Paper-Based Microfluidic Devices by Plasma Treatment. *Analytical Chemistry*, 80 (23): 9131-9134.

Li, X., Tian, J. and Shen, W. 2010. Progress in patterned paper sizing for fabrication of paper-based microfluidic sensors. *Cellulose*, 17 (3): 649-659.

Liana, D. D., Raguse, B., Gooding, J. J. and Chow, E. 2012. Recent advances in paper-based sensors. *Sensors*, 12 (9): 11505-11526.

Liang, Y., Yan, C., Guo, Q., Xu, J. and Hu, H. 2016. Spectrophotometric determination of ammonia nitrogen in water by flow injection analysis based on NH3-o-phthalaldehyde -Na2SO3 reaction. *Analytical Chemistry Research*, 10: 1-8.

Lin, Y. M., Li, L. Y., Hu, J. W., Huang, X. F., Zhou, C., Jia, M. and Li, Z. B. 2014. Photometric Determination of Ammonia Nitrogen in Slaughterhouse Wastewater with Nessler's Reagent: Effects of Different Pretreatment Methods. *Advanced Materials Research*, 955-959: 1241-1244.

Lopez-Ruiz, N., Curto, V. F., Erenas, M. M., Benito-Lopez, F., Diamond, D., Palma, A. J. and Capitan-Vallvey, L. F. 2014. Smartphone-based simultaneous pH and nitrite colorimetric determination for paper microfluidic devices. *Analytical Chemistry*, 86 (19): 9554-9562.

Lu, Y., Shi, W., Jiang, L., Qin, J. and Lin, B. 2009. Rapid prototyping of paper-based microfluidics with wax for low-cost, portable bioassay. *Electrophoresis*, 30 (9): 1497-1500.

Lu, Y., Shi, W., Qin, J. and Lin, B. 2010. Fabrication and Characterization of Paper-Based Microfluidics Prepared in Nitrocellulose Membrane By Wax Printing. *Analytical Chemistry*, 82 (1): 329-335.

Luo, X., Yan, Q., Wang, C., Luo, C., Zhou, N. and Jian, C. 2015. Treatment of Ammonia Nitrogen Wastewater in Low Concentration by Two-Stage Ozonization. *International Journal of Environmental Research and Public Health*, 12 (9): 11975-11987.

Mana, H. and Spohn, U. 2000. Rapid and selective determination of ammonium by fluorimetric flow injection analysis. *Fresenius' journal of analytical chemistry*, 366 (8): 825-829.

Martínez-Carballo, E., Sitka, A., González-Barreiro, C., Kreuzinger, N., Fürhacker, M., Scharf, S. and Gans, O. 2007. Determination of selected quaternary ammonium compounds by liquid chromatography with mass spectrometry. Part I. Application to surface, waste and indirect discharge water samples in Austria. *Environmental Pollution*, 145 (2): 489-496.

Martinez, A., Phillips, S., Carrilho, E., W Thomas, S., Sindi, H. and M Whitesides, G. 2008. *Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis*.

Martinez, A., Phillips, S., J Wiley, B., Gupta, M. and M Whitesides, G. 2009. *FLASH: A Rapid Method for Prototyping Paper-Based Microfluidic Devices*.

Martinez, A. W., Phillips, S. T., Butte, M. J. and Whitesides, G. M. 2007. Patterned Paper as a Platform for Inexpensive, Low Volume, Portable Bioassays. *Angewandte Chemie (International ed. in English)*, 46 (8): 1318-1320.

Martinez, A. W., Phillips, S. T., Whitesides, G. M. and Carrilho, E. 2010. Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices. *Analytical Chemistry*, 82 (1): 3-10.

Mason, D., Lind, A. and Marchal, G. 2002. *Statistical Techniques in Business*: México: The Mc Graw-Hill Companies.

Massart, D. L., Dijkstra, A. and Kaufman, L. 1978. *Evaluation and optimization of laboratory methods and analytical procedures*. Elsevier Scientific Pub. Co.

Mathuthu, A., Mwanga, K. and Simoro, A. 1997. Impact assessment of industrial and sewage effluents on water quality of the receiving Marimba River in Harare. *Lake Chivero: A Polluted Lake. University of Zimbabwe Publications, Harare, Zimbabwe*: 43-52.

McGuire, R. G. 1992. Reporting of objective color measurements. *HortScience*, 27 (12): 1254-1255.

Mema, V. 2010. Impact of poorly maintained waste water and sewage treatment plants: Lessons from South Africa. *ReSource*, 12 (3): 60-61.

Menesatti, P., Angelini, C., Pallottino, F., Antonucci, F., Aguzzi, J. and Costa, C. 2012. RGB color calibration for quantitative image analysis: the “3D Thin-Plate Spline” warping approach. *Sensors*, 12 (6): 7063-7079.

Meredith, N. A., Quinn, C., Cate, D. M., Reilly, T. H., Volckens, J. and Henry, C. S. 2016. Paper-based analytical devices for environmental analysis. *Analyst*, 141 (6): 1874-1887.

Meseguer-Lloret, S., Molins-Legua, C. and Campins-Falco, P. 2002. Ammonium determination in water samples by using OPA-NAC reagent: a comparative study with nessler and ammonium selective electrode methods. *International Journal of Environmental & Analytical Chemistry*, 82 (7): 475-489.

Mokrzycki, W. and Tatol, M. 2011. Colour differenceΔ E-A survey. *Machine Graphics and Vision*, 20 (4): 383-411.

Molins-Legua, C., Meseguer-Lloret, S., Moliner-Martinez, Y. and Campíns-Falcó, P. 2006. A guide for selecting the most appropriate method for ammonium determination in water analysis. *TrAC Trends in Analytical Chemistry*, 25 (3): 282-290.

Moonrungsee, N., Pencharee, S. and Jakmunee, J. 2015. Colorimetric analyzer based on mobile phone camera for determination of available phosphorus in soil. *Talanta*, 136: 204-209.

Morales-Bahnik, A., Czolk, R. and Ache, H. 1994. An optochemical ammonia sensor based on immobilized metalloporpyrins. *Sensors and Actuators B: Chemical*, 19 (1-3): 493-496.

Morbioli, G. G., Mazzu-Nascimento, T., Stockton, A. M. and Carrilho, E. 2017. Technical aspects and challenges of colorimetric detection with microfluidic paper-based analytical devices (µPADs) - A review. *Analytica Chimica Acta*, 970: 1-22.

Myers, R. L. 2007. *The 100 most important chemical compounds: a reference guide*. ABC-CLIO.

Namwong, P., Jarujamrus, P., Amatatongchai, M. and Chairam, S. 2018. Fabricating Simple Wax Screen-Printing Paper-Based Analytical Devices To Demonstrate the Concept of Limiting Reagent in Acid–Base Reactions. *Journal of Chemical Education*, 95 (2): 305-309.

Nery, E. W. and Kubota, L. T. 2013. Sensing approaches on paper-based devices: a review. *Analytical and Bioanalytical Chemistry*, 405 (24): 7573-7595.

Nie, J., Zhang, Y., Lin, L., Zhou, C., Li, S., Zhang, L. and Li, J. 2012. Low-cost fabrication of paper-based microfluidic devices by one-step plotting. *Analytical Chemistry*, 84 (15): 6331-6335.

Nordin, R., Pommen, L., Eng, P. and Meays, C. 2009. Water quality guidelines for nitrogen (nitrate, nitrite, and ammonia). *Water Stewardship Division, Ministry of Environment. Province of British Columbia*, Novotny, V. 1994. *Water quality: prevention, identification and management of diffuse pollution*. Van Nostrand-Reinhold Publishers.

Olkkonen, J., Lehtinen, K. and Erho, T. 2010. Flexographically Printed Fluidic Structures in Paper. *Analytical Chemistry*, 82 (24): 10246-10250.

Onwuegbuzie, A. J. and Leech, N. L. 2007. Validity and qualitative research: An oxymoron? *Quality & Quantity*, 41 (2): 233-249.

Organization, W. H., Research, S. P. f., Diseases, T. i. T., Diseases, W. H. O. D. o. C. o. N. T., Epidemic, W. H. O. and Alert, P. 2009. *Dengue: guidelines for diagnosis, treatment, prevention and control*. World Health Organization.

Pagans, E., Barrena, R., Font, X. and Sánchez, A. 2006. Ammonia emissions from the composting of different organic wastes. Dependency on process temperature. *Chemosphere*, 62 (9): 1534-1542.

Palenchar, M. J. 2008. Risk communication and community right to know: A public relations obligation to inform. *Advertising and Publications, School of Publications and Other Works*: 1.

Park, H.-D., Wells, G. F., Bae, H., Criddle, C. S. and Francis, C. A. 2006. Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. *Applied and environmental microbiology*, 72 (8): 5643-5647.

Patterson, R. A. 2001. Wastewater quality relationships with reuse options. *Water Science and Technology*, 43 (10): 147-154.

Patwardhan, A. 2017. *Industrial wastewater treatment*. PHI Learning Pvt. Ltd.

Pearce, K. and Ollermann, R. 1998. Status and scope of bioremediation in South Africa. *Bioremediation: Principles and Practice-Bioremediation Technologies*, 3: 155-182.

Pelton, R. 2009. Bioactive paper provides a low-cost platform for diagnostics. *TrAC Trends in Analytical Chemistry*, 28 (8): 925-942.

Phiri, O., Mumba, P., Moyo, B. and Kadewa, W. 2005. Assessment of the impact of industrial effluents on water quality of receiving rivers in urban areas of Malawi. *International Journal of Environmental Science & Technology*, 2 (3): 237-244.

Qadir, M., Wichelns, D., Raschid-Sally, L., McCornick, P. G., Drechsel, P., Bahri, A. and Minhas, P. 2010. The challenges of wastewater irrigation in developing countries. *Agricultural Water Management*, 97 (4): 561-568.

Qin, X., Gao, F. and Chen, G. 2012. Wastewater quality monitoring system using sensor fusion and machine learning techniques. *Water Research*, 46 (4): 1133-1144.

Rajaram, T. and Das, A. 2008. Water pollution by industrial effluents in India: Discharge scenarios and case for participatory ecosystem specific local regulation. *Futures*, 40 (1): 56-69.

Rajkumar, D. and Palanivelu, K. 2004. Electrochemical treatment of industrial wastewater. *Journal of Hazardous Materials*, 113 (1): 123-129.

Rice, E. W., Baird, R. B., Eaton, A. D. and Clesceri, L. S. 2012. Standard methods for the examination of water and wastewater. *Washington: APHA, AWWA, WPCR*, 1496

Ritz, C., Fairchild, B. and Lacy, M. 2004. Implications of ammonia production and emissions from commercial poultry facilities: A review. *Journal of applied poultry research*, 13 (4): 684-692.

Rodríguez, A., Zamarreño, C., Matías, I., Arregui, F., Cruz, R. and May-Arrioja, D. 2014. A Fiber Optic Ammonia Sensor Using a Universal pH Indicator. *Sensors*, 14 (3): 4060.

Romaniello, R., Leone, A. and Peri, G. 2015. Measurement of food colour in L* a* b* units from RGB digital image using least squares support vector machine regression. *Journal of Agricultural Engineering*, 46 (4): 138-143.

Roney, N. and Llados, F. 2004. Toxicological profile for ammonia.

Rowe, D. R. and Abdel-Magid, I. M. 1995. *Handbook of wastewater reclamation and reuse*. CRC Press.

Ryer-Powder, J. E. 1991. Health effects of ammonia. *Plant/operations progress*, 10 (4): 228-232.

Sackmann, E. K., Fulton, A. L. and Beebe, D. J. 2014. The present and future role of microfluidics in biomedical research. *Nature*, 507: 181.

Santhiago, M., Henry, C. S. and Kubota, L. T. 2014. Low cost, simple three dimensional electrochemical paper-based analytical device for determination of p-nitrophenol. *Electrochimica Acta*, 130: 771-777.

Sargaonkar, A. and Deshpande, V. 2003. Development of an overall index of pollution for surface water based on a general classification scheme in Indian context. *Environmental Monitoring and Assessment*, 89 (1): 43-67.

Schilling, K. M., Lepore, A. L., Kurian, J. A. and Martinez, A. W. 2012. Fully enclosed microfluidic paper-based analytical devices. *Analytical Chemistry*, 84 (3): 1579-1585.

Sharma, G., Wu, W. and Dalal, E. N. 2005. The CIEDE2000 color-difference formula: Implementation notes, supplementary test data, and mathematical observations. *Color Research & Application: Endorsed by Inter-Society Color Council, The Colour Group (Great Britain), Canadian Society for Color, Color Science Association of Japan, Dutch Society for the Study of Color, The Swedish*

Colour Centre Foundation, Colour Society of Australia, Centre Français de la Couleur, 30 (1): 21-30.

Shen, L., Hagen, J. A. and Papautsky, I. 2012. Point-of-care colorimetric detection with a smartphone. *Lab on a Chip*, 12 (21): 4240-4243.

Sheppard, L. J., Leith, I. D., Mizunuma, T., Neil Cape, J., Crossley, A., Leeson, S., Sutton, M. A., van Dijk, N. and Fowler, D. 2011. Dry deposition of ammonia gas drives species change faster than wet deposition of ammonium ions: evidence from a long-term field manipulation. *Global Change Biology*, 17 (12): 3589-3607.

Shrivastava, A. and Gupta, V. B. 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*, 2 (1): 21.

Sicard, C., Glen, C., Aubie, B., Wallace, D., Jahanshahi-Anbuhi, S., Pennings, K., Daigger, G. T., Pelton, R., Brennan, J. D. and Filipe, C. D. M. 2015. Tools for water quality monitoring and mapping using paper-based sensors and cell phones. *Water Research*, 70: 360-369.

Singh, J. and Kalamdhad, A. S. 2011. Effects of heavy metals on soil, plants, human health and aquatic life. *International journal of Research in Chemistry and Environment*, 1 (2): 15-21.

Soldat, D. J., Barak, P. and Lepore, B. J. 2009. Microscale colorimetric analysis using a desktop scanner and automated digital image analysis. *Journal of Chemical Education*, 86 (5): 617.

Songok, J. and Toivakka, M. 2016. Enhancing capillary-driven flow for paper-based microfluidic channels. *ACS applied materials & interfaces*, 8 (44): 30523-30530.

Sonune, A. and Ghate, R. 2004. Developments in wastewater treatment methods. *Desalination*, 167: 55-63.

Šrámková, I., Horstkotte, B., Sklenářová, H., Solich, P. and Kolev, S. D. 2016. A novel approach to Lab-In-Syringe Head-Space Single-Drop Microextraction and on-drop sensing of ammonia. *Analytica Chimica Acta*, 934: 132-144.

Srivastava, R., Verma, A. and Jain, V. K. 2012. Low cost, disposable colorimetric sensor for quantitative detection of ammonia gas. In: Proceedings of *16th International Workshop on Physics of Semiconductor Devices*. SPIE, 5.

Sundblad, B.-M., Larsson, B.-M., Acevedo, F., Ernstgård, L., Johanson, G., Larsson, K. and Palmberg, L. 2004. Acute respiratory effects of exposure to ammonia on healthy persons. *Scandinavian journal of work, environment & health*: 313-321.

Suneetha, M. and Ravindhranath, K. 2012. Removal of ammonia from polluted waters using biosorbents derived from powders of leaves, stems or barks of some plants. *Der Pharma Chemica*, 4 (1): 214-227.

Sutton, M., Milford, C., Dragosits, U., Place, C., Singles, R., Smith, R., Pitcairn, C., Fowler, D., Hill, J. and ApSimon, H. 1998. Dispersion, deposition and impacts of atmospheric ammonia: quantifying local budgets and spatial variability. *Environmental Pollution*, 102 (1): 349-361.

Sutton, M. A., Erisman, J. W., Dentener, F. and Möller, D. 2008. Ammonia in the environment: From ancient times to the present. *Environmental Pollution*, 156 (3): 583-604.

Swotinsky, R. B. and Chase, K. H. 1990. Health effects of exposure to ammonia: scant information. *American journal of industrial medicine*, 17 (4): 515-521.

Taudte, R. V., Beavis, A., Wilson-Wilde, L., Roux, C., Doble, P. and Blanes, L. 2013. A portable explosive detector based on fluorescence quenching of pyrene deposited on coloured wax-printed μPADs. *Lab on a Chip*, 13 (21): 4164-4172.

Tchobanoglous, G., Burton, F. L. and Stensel, H. 1991. Wastewater engineering. *Management*, 7: 1-4.

Templeton, M. R. and Butler, D. 2011. *Introduction to wastewater treatment*. Bookboon.

Tenda, K., Ota, R., Yamada, K., Henares, T. G., Suzuki, K. and Citterio, D. 2016. High-resolution microfluidic paper-based analytical devices for sub-microliter sample analysis. *Micromachines*, 7 (5): 80.

Thomas, O., Théraulaz, F., Cerdà, V., Constant, D. and Quevauviller, P. 1997. Wastewater quality monitoring. *TrAC Trends in Analytical Chemistry*, 16 (7): 419-424.

Thompson, J. and Morrison, G. 1951. *Determination of Organic Nitrogen. Control of Variables in the Use of Nessler's Reagent*.

Thuo, M. M., Martinez, R. V., Lan, W.-J., Liu, X., Barber, J., Atkinson, M. B., Bandarage, D., Bloch, J.-F. and Whitesides, G. M. 2014. Fabrication of low-cost paper-based microfluidic devices by embossing or cut-and-stack methods. *Chemistry of Materials*, 26 (14): 4230-4237.

Tilche, A. and Orhon, D. 2002. Appropriate basis of effluent standards for industrial wastewaters. *Water Science and Technology*, 45 (12): 1-11.

Timmer, B., Olthuis, W. and Berg, A. v. d. 2005. Ammonia sensors and their applications—a review. *Sensors and Actuators B: Chemical*, 107 (2): 666-677.

Tkalcic, M. and Tasic, J. F. 2003. Colour spaces: perceptual, historical and applicational background. In: Proceedings of *The IEEE Region 8 EUROCON 2003. Computer as a Tool.* 22-24 Sept. 2003. 304-308 vol.301.

Tomazelli Coltro, W. K., Cheng, C. M., Carrilho, E. and de Jesus, D. P. 2014. Recent advances in low-cost microfluidic platforms for diagnostic applications. *Electrophoresis*, 35 (16): 2309-2324.

Tominaga, S. 1992. Color classification of natural color images. *Color Research & Application*, 17 (4): 230-239.

Ujang, Z. and Henze, M. 2006. *Municipal wastewater management in developing countries*. IWA Publishing.

Verdouw, H., Van Echteld, C. J. A. and Dekkers, E. M. J. 1978. Ammonia determination based on indophenol formation with sodium salicylate. *Water Research*, 12 (6): 399-402.

Wahyuni, D. 2012. The research design maze: Understanding paradigms, cases, methods and methodologies.

Weatherall, I. L. and Coombs, B. D. 1992. Skin color measurements in terms of CIELAB color space values. *Journal of investigative dermatology*, 99 (4): 468-473.

Weatherburn, M. 1967. Phenol-hypochlorite reaction for determination of ammonia. *Analytical Chemistry*, 39 (8): 971-974.

Welch, A. 2006. Exposing the dangers of anhydrous ammonia. *The Nurse Practitioner*, 31 (11): 40-45.

Wiersma, W. and Jurs, S. G. 2005. Research methods in education: An introduction.

Willason, S. and Johnson, K. 1986. A rapid, highly sensitive technique for the determination of ammonia in seawater. *Marine Biology*, 91 (2): 285-290.

Wu, H. Z. and Cao, A. 2013. Preparation and Adding Methods of Nessler's Reagent Having Effects on Determination of Water Quality Ammonia Nitrogen. *Advanced Materials Research*, 726-731: 1362-1366.

Xia, Y., Si, J. and Li, Z. 2016. Fabrication techniques for microfluidic paper-based analytical devices and their applications for biological testing: A review. *Biosensors and Bioelectronics*, 77: 774-789.

Yager, P., Edwards, T., Fu, E., Helton, K., Nelson, K., Tam, M. R. and Weigl, B. H. 2006. Microfluidic diagnostic technologies for global public health. *Nature*, 442 (7101): 412.

Yakoh, A., Rattanarat, P., Siangproh, W. and Chailapakul, O. 2018. Simple and selective paper-based colorimetric sensor for determination of chloride ion in environmental samples using label-free silver nanoprisms. *Talanta*, 178: 134-140.

Yamada, K., Henares, T. G., Suzuki, K. and Citterio, D. 2015. Paper-based inkjet-printed microfluidic analytical devices. *Angewandte Chemie International Edition*, 54 (18): 5294-5310.

Yang, H. and Cheng, H. 2007. Controlling nitrite level in drinking water by chlorination and chloramination. *Separation and Purification Technology*, 56 (3): 392-396.

Yang, Y., Noviana, E., Nguyen, M. P., Geiss, B. J., Dandy, D. S. and Henry, C. S. 2016. based microfluidic devices: Emerging themes and applications. *Analytical Chemistry*, 89 (1): 71-91.

Yetisen, A. K., Akram, M. S. and Lowe, C. R. 2013. based microfluidic point-of-care diagnostic devices. *Lab on a Chip*, 13 (12): 2210-2251.

Yu, J., Ge, L., Huang, J., Wang, S. and Ge, S. 2011. Microfluidic paper-based chemiluminescence biosensor for simultaneous determination of glucose and uric acid. *Lab on a Chip*, 11 (7): 1286-1291.

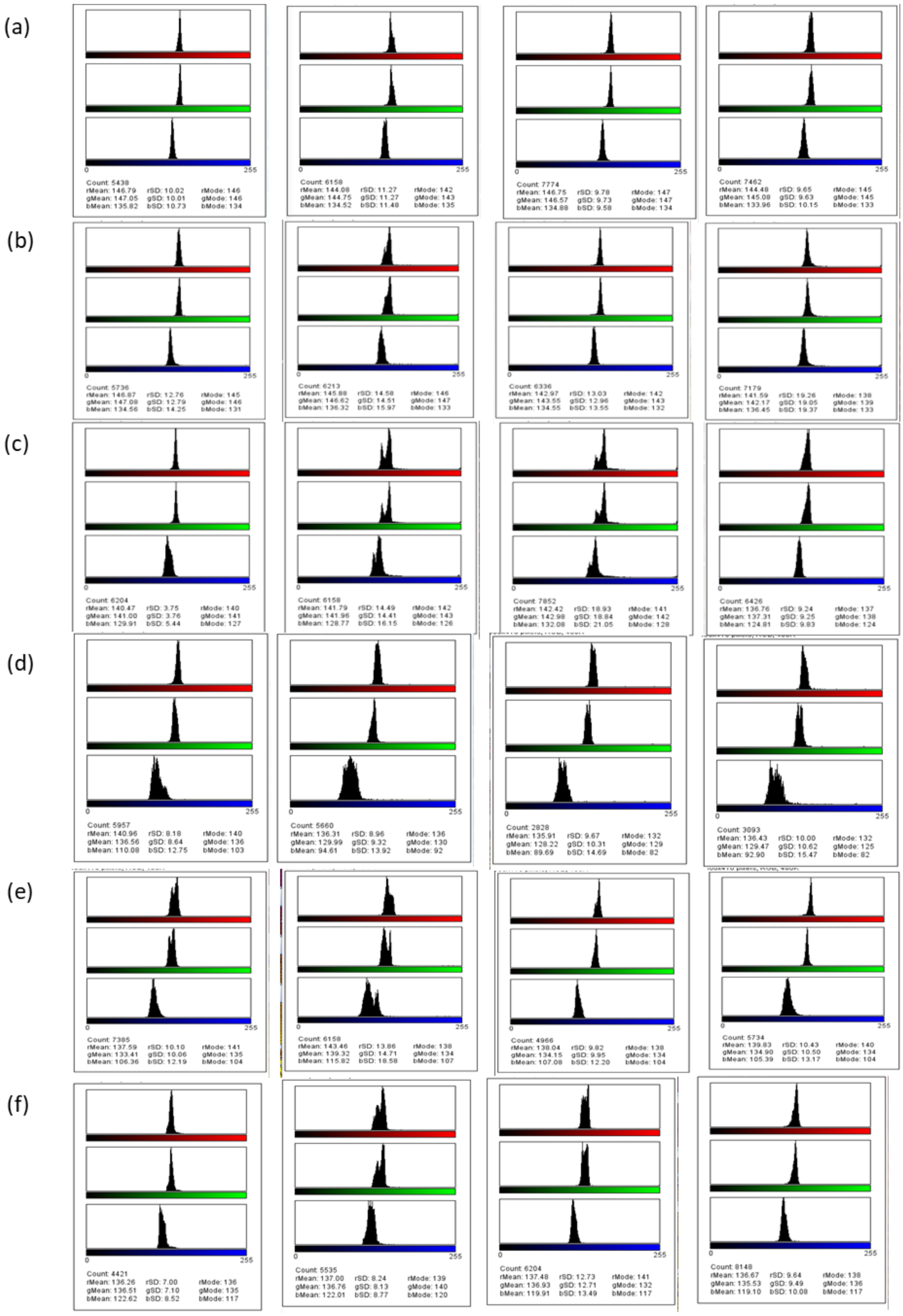
Zadorojny, C., Saxton, S. and Finger, R. 1973. Spectrophotometric determination of ammonia. *Journal (Water Pollution Control Federation)*: 905-912.

Zhang, X. and Wandell, B. A. 1996. A spatial extension of CIELAB for digital color image reproduction. In: Proceedings of *SID international symposium digest of technical papers*. Citeseer, 731-734.

Zhou, L. and Boyd, C. E. 2016. Comparison of Nessler, phenate, salicylate and ion selective electrode procedures for determination of total ammonia nitrogen in aquaculture. *Aquaculture*, 450: 187-193.

Zhu, Z., Lu, J. J., Almeida, M. I. G., Pu, Q., Kolev, S. D. and Liu, S. 2015. A microfabricated electroosmotic pump coupled to a gas-diffusion microchip for flow injection analysis of ammonia. *Microchimica Acta*, 182 (5-6): 1063-1070.

ANNEXURES



```
Count: 5438
rMean: 146.79   rSD: 10.02   rMode: 146
gMean: 147.05   gSD: 10.01   gMode: 146
bMean: 135.82   bSD: 10.73   bMode: 134
```

```
Count: 6158
rMean: 144.08   rSD: 11.27   rMode: 142
gMean: 144.75   gSD: 11.27   gMode: 143
bMean: 134.52   bSD: 11.48   bMode: 135
```

```
Count: 7774
rMean: 146.75   rSD: 9.78    rMode: 147
gMean: 146.57   gSD: 9.73    gMode: 147
bMean: 134.88   bSD: 9.58    bMode: 134
```

```
Count: 7462
rMean: 144.48   rSD: 9.65    rMode: 145
gMean: 145.08   gSD: 9.63    gMode: 145
bMean: 133.96   bSD: 10.15   bMode: 133
```

```
Count: 5736
rMean: 146.87   rSD: 12.76   rMode: 145
gMean: 147.08   gSD: 12.79   gMode: 146
bMean: 134.56   bSD: 14.25   bMode: 131
```

```
Count: 6213
rMean: 145.88   rSD: 14.58   rMode: 146
gMean: 146.62   gSD: 14.51   gMode: 147
bMean: 136.32   bSD: 15.97   bMode: 133
```

```
Count: 6336
rMean: 142.97   rSD: 13.03   rMode: 142
gMean: 143.55   gSD: 12.96   gMode: 143
bMean: 134.55   bSD: 13.55   bMode: 132
```

```
Count: 7179
rMean: 141.59   rSD: 19.26   rMode: 138
gMean: 142.17   gSD: 19.05   gMode: 139
bMean: 136.45   bSD: 19.37   bMode: 133
```

```
Count: 6204
rMean: 140.47   rSD: 3.75    rMode: 140
gMean: 141.00   gSD: 3.76    gMode: 141
bMean: 129.91   bSD: 5.44    bMode: 127
```

```
Count: 6158
rMean: 141.79   rSD: 14.49   rMode: 142
gMean: 141.96   gSD: 14.41   gMode: 143
bMean: 128.77   bSD: 16.15   bMode: 126
```

```
Count: 7852
rMean: 142.42   rSD: 18.93   rMode: 141
gMean: 142.98   gSD: 18.84   gMode: 142
bMean: 132.08   bSD: 21.05   bMode: 128
```

```
Count: 6426
rMean: 136.76   rSD: 9.24    rMode: 137
gMean: 137.31   gSD: 9.25    gMode: 138
bMean: 124.81   bSD: 9.83    bMode: 124
```

```
Count: 5957
rMean: 140.96   rSD: 8.18    rMode: 140
gMean: 136.56   gSD: 8.64    gMode: 136
bMean: 110.08   bSD: 12.75   bMode: 103
```

```
Count: 5660
rMean: 136.31   rSD: 8.96    rMode: 136
gMean: 129.99   gSD: 9.32    gMode: 130
bMean: 94.61    bSD: 13.92   bMode: 92
```

```
Count: 2828
rMean: 135.91   rSD: 9.67    rMode: 132
gMean: 128.22   gSD: 10.31   gMode: 129
bMean: 89.69    bSD: 14.69   bMode: 82
```

```
Count: 3093
rMean: 136.43   rSD: 10.00   rMode: 132
gMean: 129.47   gSD: 10.62   gMode: 125
bMean: 92.90    bSD: 15.47   bMode: 82
```

```
Count: 7385
rMean: 137.59   rSD: 10.10   rMode: 141
gMean: 133.41   gSD: 10.06   gMode: 135
bMean: 106.36   bSD: 12.19   bMode: 104
```

```
Count: 6158
rMean: 143.46   rSD: 13.86   rMode: 138
gMean: 139.32   gSD: 14.71   gMode: 134
bMean: 115.82   bSD: 18.58   bMode: 107
```

```
Count: 4966
rMean: 138.04   rSD: 9.82    rMode: 138
gMean: 134.15   gSD: 9.95    gMode: 134
bMean: 107.08   bSD: 12.20   bMode: 104
```

```
Count: 5734
rMean: 139.83   rSD: 10.43   rMode: 140
gMean: 134.90   gSD: 10.50   gMode: 134
bMean: 105.39   bSD: 13.17   bMode: 104
```

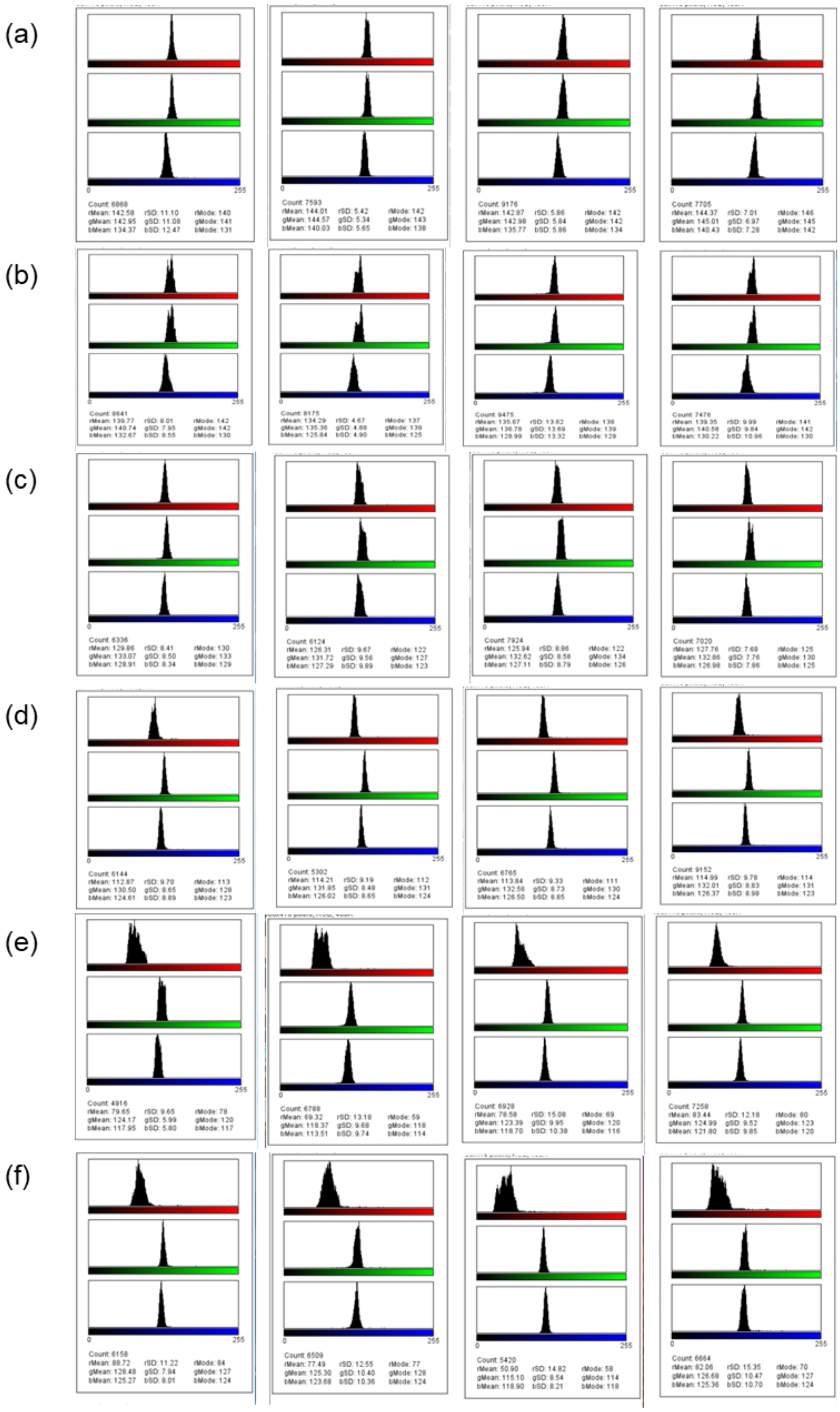
```
Count: 4421
rMean: 136.26   rSD: 7.00    rMode: 136
gMean: 136.51   gSD: 7.10    gMode: 135
bMean: 122.62   bSD: 8.52    bMode: 117
```

```
Count: 5535
rMean: 137.00   rSD: 8.24    rMode: 139
gMean: 136.76   gSD: 8.13    gMode: 140
bMean: 122.01   bSD: 8.77    bMode: 120
```

```
Count: 6204
rMean: 137.48   rSD: 12.73   rMode: 141
gMean: 136.93   gSD: 12.71   gMode: 132
bMean: 119.91   bSD: 13.49   bMode: 117
```

```
Count: 8148
rMean: 136.67   rSD: 9.64    rMode: 138
gMean: 135.53   gSD: 9.49    gMode: 136
bMean: 119.10   bSD: 10.08   bMode: 117
```

***Annexure 1** RGB color intensity of quadruplicate ammonia standards where (a) is 0 ppm, (b) 0.1 ppm , (c) 0.5 ppm, (d) 1 ppm, (e) 3 ppm and (f) 5 ppm using Nessler reagent method*



```
(a)
Count: 6868
rMean: 142.58   rSD: 11.10   rMode: 140
gMean: 142.95   gSD: 11.08   gMode: 141
bMean: 134.37   bSD: 12.47   bMode: 131

Count: 7593
rMean: 144.01   rSD: 5.42    rMode: 142
gMean: 144.57   gSD: 5.34    gMode: 143
bMean: 140.03   bSD: 5.65    bMode: 138

Count: 9176
rMean: 142.87   rSD: 5.86    rMode: 142
gMean: 142.98   gSD: 5.84    gMode: 142
bMean: 135.77   bSD: 5.86    bMode: 134

Count: 7705
rMean: 144.37   rSD: 7.01    rMode: 146
gMean: 145.01   gSD: 6.97    gMode: 145
bMean: 140.43   bSD: 7.28    bMode: 142
```

```
(b)
Count: 8641
rMean: 139.77   rSD: 8.01    rMode: 142
gMean: 140.74   gSD: 7.95    gMode: 142
bMean: 132.67   bSD: 8.55    bMode: 130

Count: 8175
rMean: 134.29   rSD: 4.67    rMode: 137
gMean: 135.36   gSD: 4.88    gMode: 139
bMean: 125.84   bSD: 4.90    bMode: 125

Count: 9475
rMean: 135.67   rSD: 13.62   rMode: 138
gMean: 136.78   gSD: 13.69   gMode: 139
bMean: 128.99   bSD: 13.32   bMode: 129

Count: 7476
rMean: 139.35   rSD: 9.99    rMode: 141
gMean: 140.58   gSD: 9.84    gMode: 142
bMean: 130.22   bSD: 10.96   bMode: 130
```

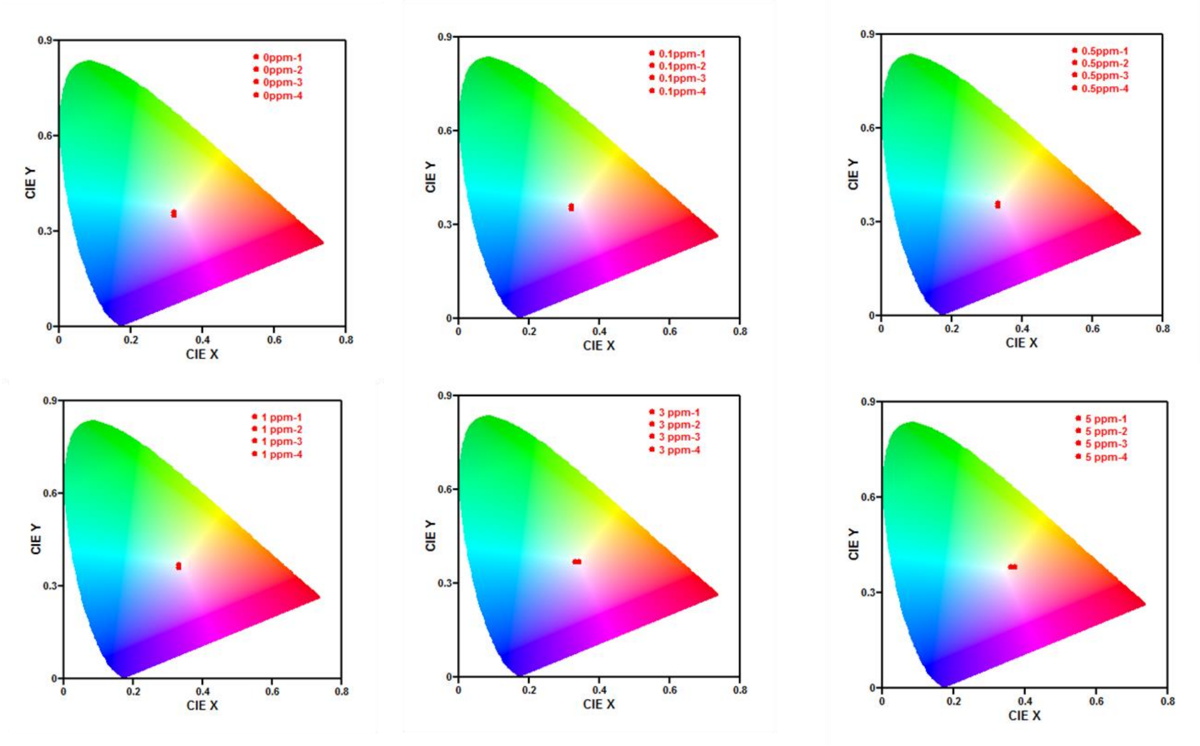
```
(c)
Count: 6336
rMean: 129.86   rSD: 8.41    rMode: 130
gMean: 133.07   gSD: 8.50    gMode: 133
bMean: 128.91   bSD: 8.34    bMode: 129

Count: 6124
rMean: 126.31   rSD: 9.67    rMode: 122
gMean: 131.72   gSD: 9.56    gMode: 127
bMean: 127.29   bSD: 9.89    bMode: 123

Count: 7924
rMean: 125.94   rSD: 8.86    rMode: 122
gMean: 132.62   gSD: 8.58    gMode: 134
bMean: 127.11   bSD: 8.79    bMode: 126

Count: 7020
rMean: 127.76   rSD: 7.68    rMode: 125
gMean: 132.86   gSD: 7.76    gMode: 130
bMean: 126.98   bSD: 7.86    bMode: 125
```

```
(d)
Count: 6144
rMean: 112.87   rSD: 9.70    rMode: 113
gMean: 130.50   gSD: 8.65    gMode: 128
bMean: 124.61   bSD: 8.89    bMode: 123

Count: 5302
rMean: 114.21   rSD: 9.19    rMode: 112
gMean: 131.85   gSD: 8.48    gMode: 131
bMean: 126.02   bSD: 8.65    bMode: 124

Count: 6765
rMean: 113.84   rSD: 9.33    rMode: 111
gMean: 132.56   gSD: 8.73    gMode: 130
bMean: 126.50   bSD: 8.85    bMode: 124

Count: 9152
rMean: 114.99   rSD: 9.78    rMode: 114
gMean: 132.01   gSD: 8.83    gMode: 131
bMean: 126.37   bSD: 8.98    bMode: 123
```

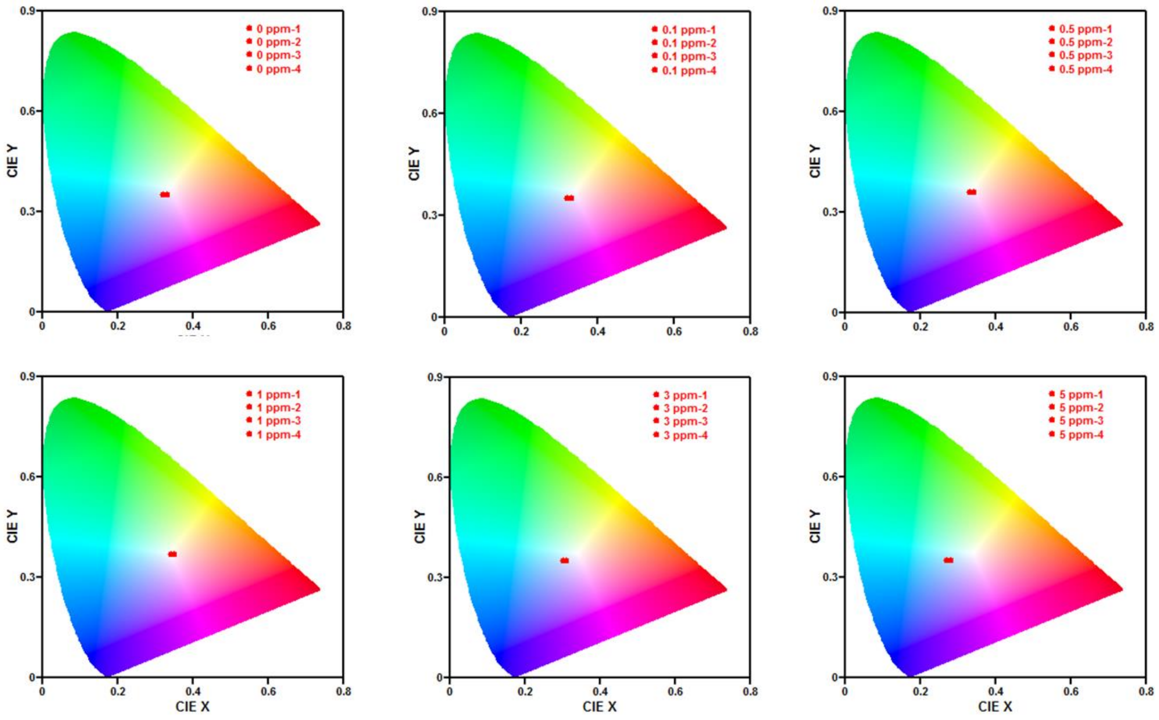
```
(e)
Count: 4916
rMean: 79.65    rSD: 9.65    rMode: 78
gMean: 124.17   gSD: 5.99    gMode: 120
bMean: 117.95   bSD: 5.80    bMode: 117

Count: 6788
rMean: 69.32    rSD: 13.18   rMode: 59
gMean: 118.37   gSD: 9.68    gMode: 118
bMean: 113.51   bSD: 9.74    bMode: 114

Count: 6928
rMean: 78.58    rSD: 15.08   rMode: 69
gMean: 123.39   gSD: 9.95    gMode: 120
bMean: 118.70   bSD: 10.38   bMode: 116

Count: 7258
rMean: 83.44    rSD: 12.18   rMode: 80
gMean: 124.99   gSD: 9.52    gMode: 123
bMean: 121.80   bSD: 9.85    bMode: 120
```

```
(f)
Count: 6158
rMean: 88.72    rSD: 11.22   rMode: 84
gMean: 128.48   gSD: 7.94    gMode: 127
bMean: 125.27   bSD: 8.01    bMode: 124

Count: 6509
rMean: 77.49    rSD: 12.55   rMode: 77
gMean: 125.30   gSD: 10.40   gMode: 128
bMean: 123.68   bSD: 10.36   bMode: 124

Count: 5420
rMean: 50.90    rSD: 14.82   rMode: 58
gMean: 115.10   gSD: 8.54    gMode: 114
bMean: 118.90   bSD: 8.21    bMode: 118

Count: 6664
rMean: 82.06    rSD: 15.35   rMode: 70
gMean: 126.68   gSD: 10.47   gMode: 127
bMean: 125.36   bSD: 10.70   bMode: 124
```

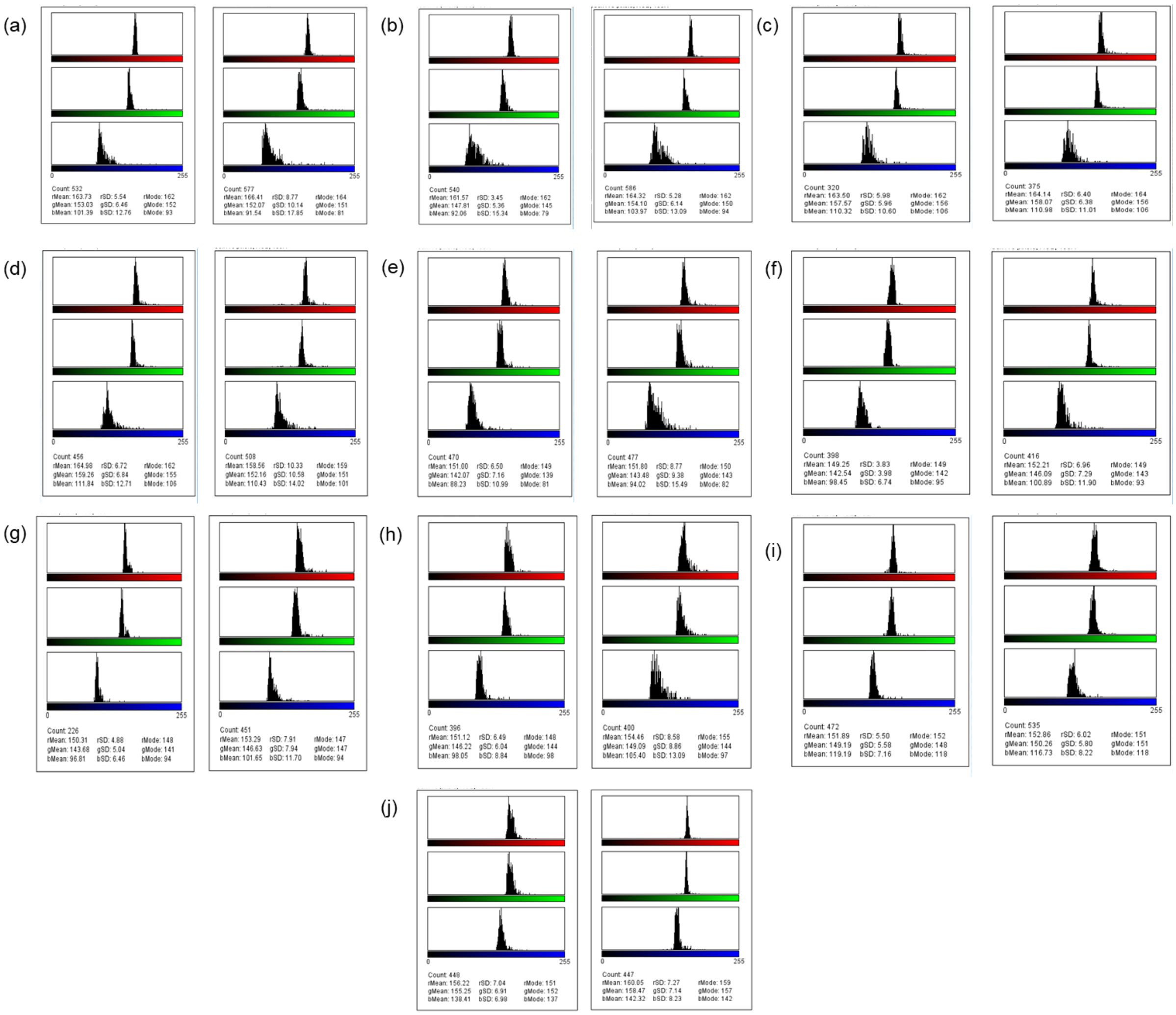
Annexure 2 *RGB color intensity of quadruplicate ammonia standards where (a) is 0 ppm, (b) 0.1 ppm, (c) 0.5 ppm, (d) 1 ppm, (e) 3 ppm and (f) 5 ppm using Salicylate method*



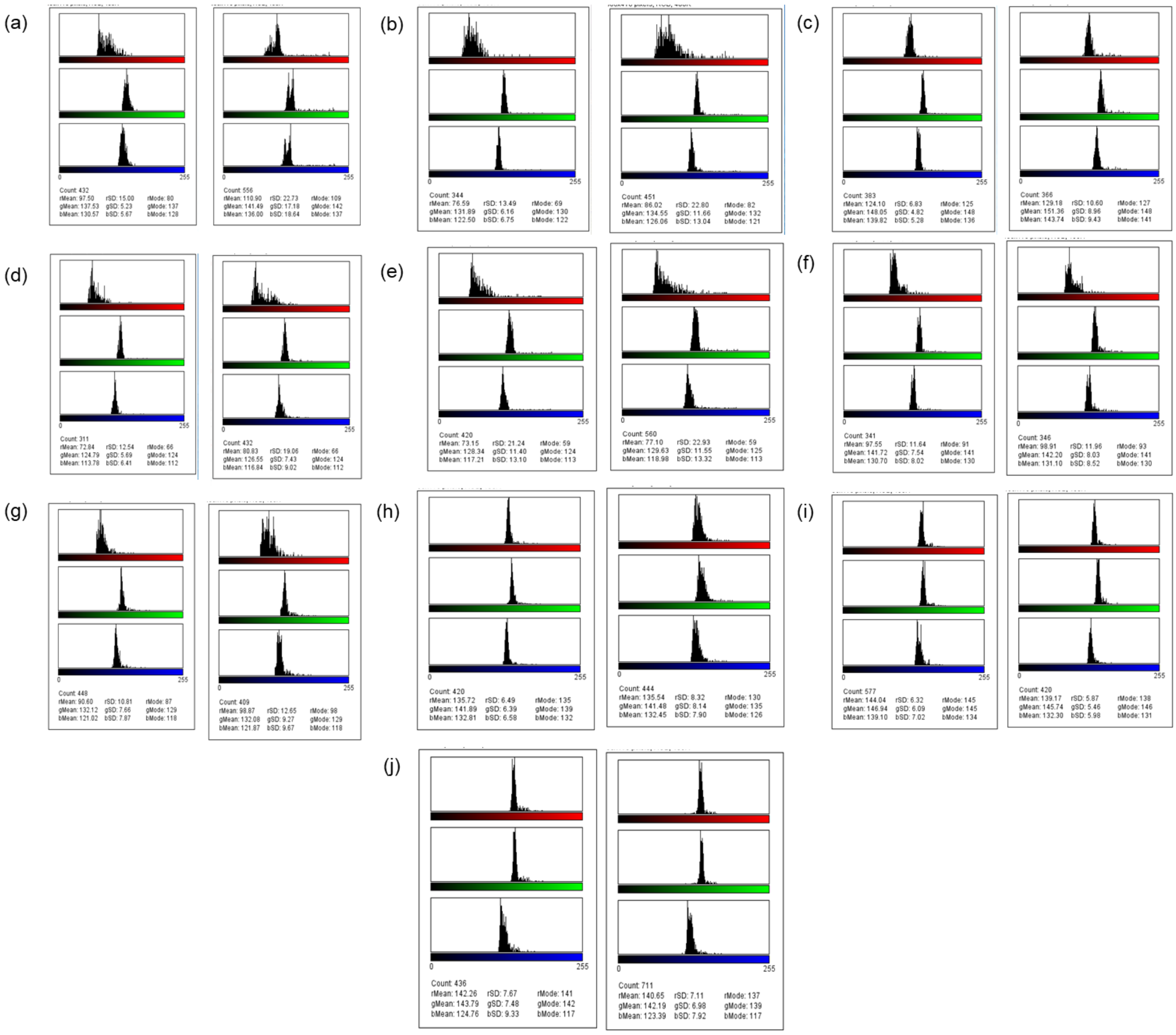
***Annexure 3** chromaticity diagram for ammonia standards (0-5 ppm) with the Nessler reagent*



***Annexure 4** Chromaticity diagram for ammonia standards with salicylate method*



Annexure 5 *RGB colour intensity of the 10 days wastewater samples (a) day 1, (b) day 2, (c) day 3, (d) day 4, (e) day 5, (f) day 6, (g) day 7, (h) day 8, (i) day 9 and (j) day 10 analysed using the Nessler reagent method*



```
Count: 432
rMean: 97.50    rSD: 15.00    rMode: 80
gMean: 137.53   gSD: 5.23     gMode: 137
bMean: 130.57   bSD: 5.67     bMode: 128
```

```
Count: 556
rMean: 110.90   rSD: 22.73    rMode: 109
gMean: 141.49   gSD: 17.18    gMode: 142
bMean: 136.00   bSD: 18.64    bMode: 137
```

```
Count: 344
rMean: 76.59    rSD: 13.49    rMode: 69
gMean: 131.89   gSD: 6.16     gMode: 130
bMean: 122.50   bSD: 6.75     bMode: 122
```

```
Count: 451
rMean: 86.02    rSD: 22.80    rMode: 82
gMean: 134.55   gSD: 11.66    gMode: 132
bMean: 126.06   bSD: 13.04    bMode: 121
```

```
Count: 383
rMean: 124.10   rSD: 6.83     rMode: 125
gMean: 148.05   gSD: 4.82     gMode: 148
bMean: 139.82   bSD: 5.28     bMode: 136
```

```
Count: 366
rMean: 129.18   rSD: 10.60    rMode: 127
gMean: 151.36   gSD: 8.96     gMode: 148
bMean: 143.74   bSD: 9.43     bMode: 141
```

```
Count: 311
rMean: 72.84    rSD: 12.54    rMode: 66
gMean: 124.79   gSD: 5.69     gMode: 124
bMean: 113.78   bSD: 6.41     bMode: 112
```

```
Count: 432
rMean: 80.83    rSD: 19.06    rMode: 66
gMean: 126.55   gSD: 7.43     gMode: 124
bMean: 116.84   bSD: 9.02     bMode: 112
```

```
Count: 420
rMean: 73.15    rSD: 21.24    rMode: 59
gMean: 128.34   gSD: 11.40    gMode: 124
bMean: 117.21   bSD: 13.10    bMode: 113
```

```
Count: 560
rMean: 77.10    rSD: 22.93    rMode: 59
gMean: 129.63   gSD: 11.55    gMode: 125
bMean: 118.98   bSD: 13.32    bMode: 113
```

```
Count: 341
rMean: 97.55    rSD: 11.64    rMode: 91
gMean: 141.72   gSD: 7.54     gMode: 141
bMean: 130.70   bSD: 8.02     bMode: 130
```

```
Count: 346
rMean: 98.91    rSD: 11.96    rMode: 93
gMean: 142.20   gSD: 8.03     gMode: 141
bMean: 131.10   bSD: 8.52     bMode: 130
```

```
Count: 448
rMean: 90.60    rSD: 10.81    rMode: 87
gMean: 132.12   gSD: 7.66     gMode: 129
bMean: 121.02   bSD: 7.87     bMode: 118
```

```
Count: 409
rMean: 98.87    rSD: 12.65    rMode: 98
gMean: 132.08   gSD: 9.27     gMode: 129
bMean: 121.87   bSD: 9.67     bMode: 118
```

```
Count: 420
rMean: 135.72   rSD: 6.49     rMode: 135
gMean: 141.89   gSD: 6.39     gMode: 139
bMean: 132.81   bSD: 6.58     bMode: 132
```

```
Count: 444
rMean: 135.54   rSD: 8.32     rMode: 130
gMean: 141.48   gSD: 8.14     gMode: 135
bMean: 132.45   bSD: 7.90     bMode: 126
```

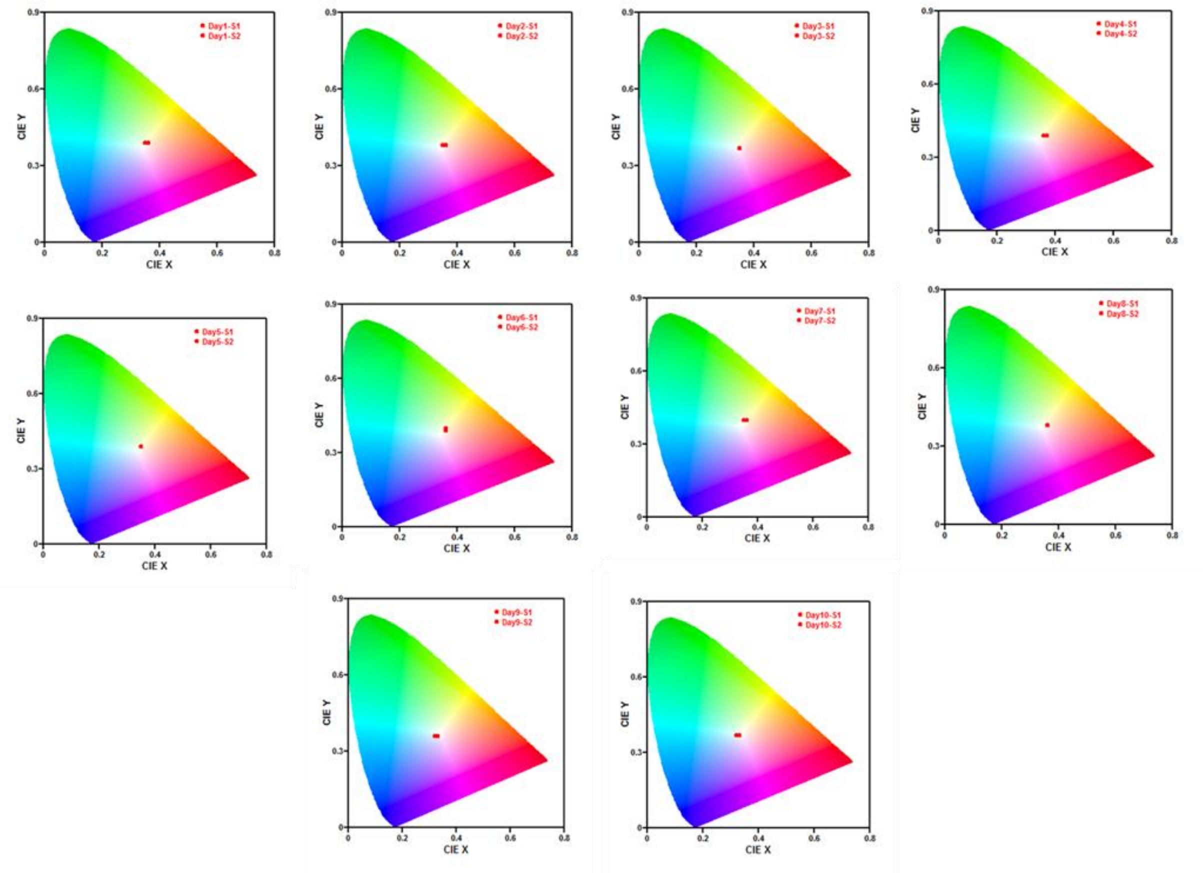
```
Count: 577
rMean: 144.04   rSD: 6.32     rMode: 145
gMean: 146.94   gSD: 6.09     gMode: 145
bMean: 139.10   bSD: 7.02     bMode: 134
```

```
Count: 420
rMean: 139.17   rSD: 5.87     rMode: 138
gMean: 145.74   gSD: 5.46     gMode: 146
bMean: 132.30   bSD: 5.98     bMode: 131
```

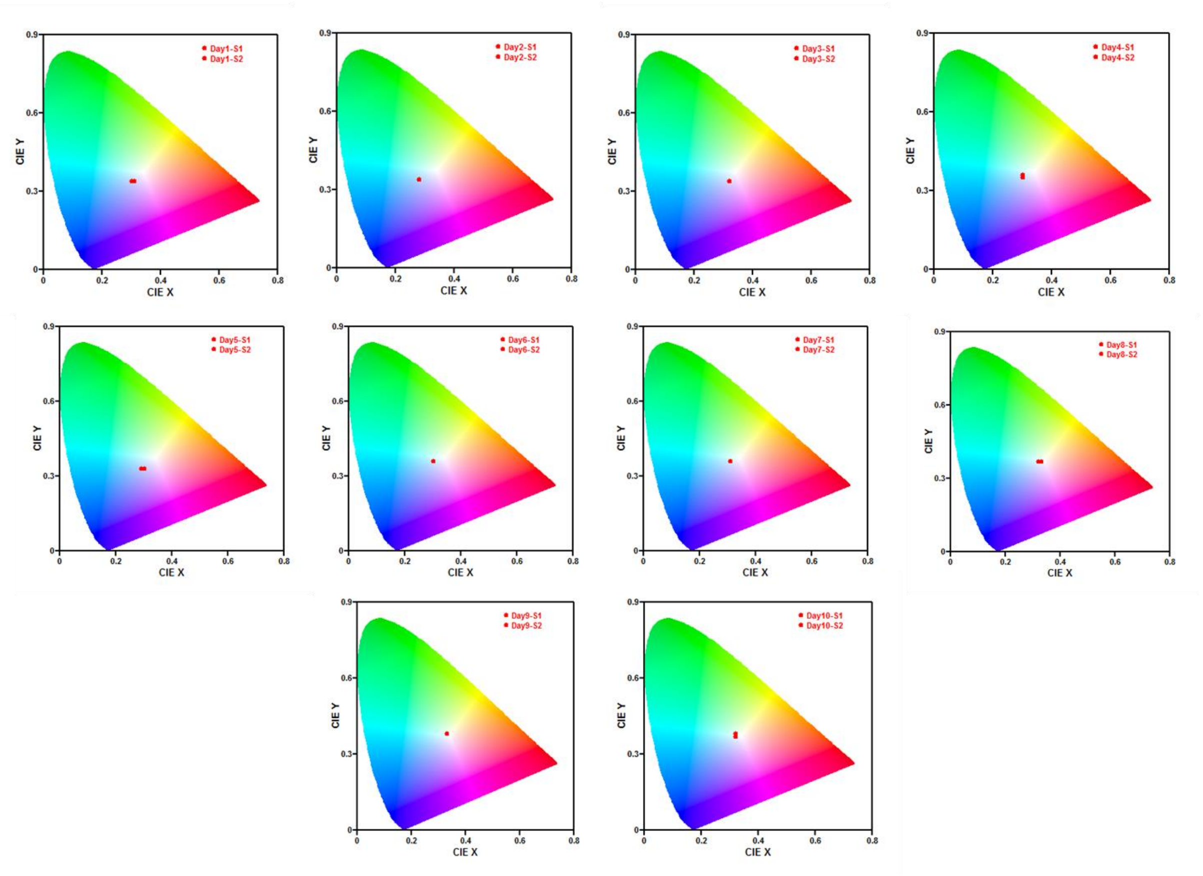
```
Count: 436
rMean: 142.26   rSD: 7.67     rMode: 141
gMean: 143.79   gSD: 7.48     gMode: 142
bMean: 124.76   bSD: 9.33     bMode: 117
```

```
Count: 711
rMean: 140.65   rSD: 7.11     rMode: 137
gMean: 142.19   gSD: 6.98     gMode: 139
bMean: 123.39   bSD: 7.92     bMode: 117
```

Annexure 6 *RGB colour intensity of the 10 days wastewater samples (a) day 1, (b) day 2, (c) day 3, (d) day 4, (e) day 5, (f) day 6, (g) day 7, (h) day 8, (i) day 9 and (j) day 10 analysed using the Salicylate method*



Annexure 7 *CIELAB chromaticity diagram of wastewater samples for Nessler reagent method*



Annexure 8 *CIELAB chromaticity diagram of wastewater samples for salicylate method*