Emerging and Persistent Contaminants/Pathogens: Monitoring Methods Development

Report to the
Water Research Commission

by

L.F. Petrik, O.O. Fatoba, G.M. Ndilowe, E.O. Omoniyi, D. Chimponda and C.A. Bode-Aluko

Environmental and Nano Sciences Research Group, Chemistry Department, University of the Western Cape, Bellville, South Africa

WRC Report No 2521/1/19
ISBN 978-0-6392-0102-3

February 2020

WATER
RESEARCH
COMMISSION

Obtainable from

Water Research Commission

Private Bag X03

GEZINA, 0031

orders@wrc.org.za or download from www.wrc.org.za

EXECUTIVE SUMMARY

There are several conventional analytical techniques which are well established for analysis of water pollutants such as high-performance liquid chromatography (HPLC), gas chromatography and liquid chromatography coupled with mass spectrometry (GC-MS, LC-MS), electrochemical and spectrophotometry. These techniques require complex sample preparation processes and lack sensitivity and recognition capacity for quantifying different persistent organic pollutants. Although such pollutants can be detected, it has been found to be difficult to identify or quantify them at low concentrations. These limitations have led to the exploration of novel detection technologies such as biosensors, fluorescence probes, surface-enhanced Raman spectroscopy (SERS), etc. with the aim of detecting very low concentrations of diverse pollutants. Among the emerging techniques, SERS has attracted considerable attention in the detection of trace level analytes in chemical analysis and biochemistry. SERS, an extension to Raman spectroscopy, is an analytical technique based on molecular vibrations. SERS relies on a combination of electromagnetic and chemical enhancement mechanisms that involve surface electron movement in the SERS-active material and a charge transfer between the SERS-active material and the analyte of interest. Electromagnetic enhancements arise from excitement of the surface plasmon (oscillating electron wave) of the SERS-active material that has been induced by an incident laser, thereby creating an electromagnetic field. The energy from the surface plasmon is then absorbed by a nearby, or an adsorbed, analyte molecule, which then transfers the energy back to the SERS-active material less the amount transferred to its nuclei. SERS has several analytical advantages over other methods, including ultra-sensitivity and inherent molecular specificity. The technique requires little or no sample preparation apart from separation, and is a convenient and cost-effective process for development of miniaturised platforms for point of use equipment. Furthermore, it can be directly used for water sample analysis with negligible background noise from the aqueous matrix. The performance of SERS is based on detection sensitivity, which depends on the surface properties of the SERS active materials

Surface Enhanced Raman spectroscopy (SERS) with a narrow spectral band is considered as a powerful analytical tool for detection of pollutants in a complex mixture. However, the performance of SERS as a detection tool depends on the nature of the platform upon which the SERS analysis is carried out. The aim of this study was to develop nanosilver coated track-etched membranes as platforms that could be used in Surface Enhanced Raman spectroscopy (SERS). The objectives were the identification of emerging micropollutants in water samples, and comparison of the new analytical method with existing methods in order to determine its advantages.

The detection and monitoring technique considered in this study comprised of a pre-concentration step followed by SERS. The pre-concentration step involved utilisation of silver nanoparticles supported upon track-etch membrane. In this study, novel surface enhanced Raman Spectroscopy (SERS)-active platforms containing arrays of silver nanoparticles on the surface of track-etch polyterephthalate membranes were developed. The Ag nanoparticles were anchored on polymer or quartz supports using self-assembled silver monolayers containing thiolated organic molecules. The self-assembled-monolayer modified SERS membrane was used for the detection of emerging and persistent contaminants in model solutions as well as in real waters. The pre-concentration step consisted of separation of the contaminants from the aqueous matrix by

rapid filtration through small nano sized pores in the track etched membrane to concentrate the dilute pollutants onto the surface of the platform. The detection step focused on improving the detection sensitivity of SERS by tailoring the surface enhanced Ag nanostructures supported on the track membrane platform to detect emerging pollutants at low concentrations.

The study covered in detail the results and characterisation of silver-coated track-etched polyethene terephthalate (PET) membrane used as a platform for the detection of acetaminophen by SERS. Firstly, the proof of concept of using the track membrane filter as a rapid separator for capturing and pre-concentration of the contaminants was proved feasible, but some analyte loss occurred through the sub-micron sized pores of the trackmembrane. Thus, smaller track membrane pores would be needed to retain more of the analyte in question. However, the pre-concentration step through the track membrane did not allow separation of different analytes. The pores in track membranes can be tailored to the very small nanometre diameter, thus it is recommended that the future work on SERS platforms consider different track membrane pore sizes for adequate analyte retention, with prior separation of mixtures. Surface-enhanced Raman spectroscopy requires metal nanostructures to enhance the weak Raman signals of the analytes and silver nanoparticles were successfully used in the study to enhance the Raman signal of selected analytes, once again proving the concept and versatility of using plasmon decorated track membrane platforms for rapid analyte detection. A quartz platform was used as control to compare the detection of selected pollutants using the surface-enhanced silver nanoparticles on the silver-coated track-etched PET membrane, which was also compared with unmodified track-etched PET membranes. Silver nanoparticles were found to significantly enhance the Raman signal of analytes and specific analyte peaks could be used for identification and quantification. Further Raman characterisation of the PET membranes that were decorated with silver nanoparticles for different times showed that the silver nanoparticle coating successfully suppressed the background Raman signal of the polyethene terephthalate polymer support leading to greater sensitivity towards detecting the pollutant molecules due to the plasmon resonance of the Ag nanoparticles. The study also made a comparison of various concentrations of a selected contaminant, acetaminophen, which showed linear increases in some peak heights of the Raman spectra with an increase in concentration of the analyte. The peak intensity trend for the C-O (861) Raman vibration of acetominophen showed a linear response, allowing quantification. The peak intensity of the C-O bond had the best correlation with the concentration with excellent linearity. It is important to note that complex mixtures of persistent organic pollutants would need separation prior to identification and quantification by the SERS platform, thus it is recommended that the SERS platform should be connected to a separation system such as capillary electrophoresis in future studies. This new SERS platform for the Raman spectroscopic method can be considered suitable for rapid screening and sensing the presence of contaminants in environmental samples that require low detection limits, as well as offering fingerprinting capability when coupled to a suitable separation technique. It is proposed that capillary electrophoresis would be a suitable separation technique to couple to the novel plasmon silver enhanced PET based SERS platform for rapid identification and quantification to become possible.

A cost comparison of the different analytical techniques is provided in the Appendix, based on small scale experimental costs, which may be different once scaled up.

ACKNOWLEDGEMENTS

The project team wishes to thank the following people for their contributions to the project.

Reference Group	**Affiliation**
Dr E Ubomba-Jaswa	Water Research Commission
Dr B Shaddock	Golder Associates Research Laboratory (Pty) Ltd. (GARL)
Dr B Delcarme	Cape Peninsula University of Technology
Dr J Chamier	Council for Scientific and Industrial Research
Prof S Dube	University of South Africa (UNISA)
Prof MM Nindi	University of South Africa (UNISA)
Mr JN Rossouw	Aurecon
Mr CD Swartz	Chris Swartz Water Utilization Engineers

This page was intentionally left blank

CONTENTS

LIST OF FIGURES

LIST OF TABLES

PUBLICATIONS AND CONFERENCES

ARTICLES

Published:

Syrtsova, D. A., Teplyakov, V. V., Kochnev, Y. K., Nechaev , A. N., Apel, P. Y., Adeniyi, O. R. And Petrik, L. (2016). Structure and properties of poly(4-methylpentene-1) track-etched membranes. Petroleum Chemistry, 56(4), pp. 294-302. DOI: 10.1134/S0965544116040113

Rossouw, A., Artoshina, O. V., Nechaev, A. N., Apel, P. Yu., Petrik, L., Perold, W. J., Pineda-Vargas, C. A. (2015). Stable Ion Beam Analysis (RBS and PIXE) Study of Photocatalytic Track-Etched Membranes. Exotic Nuclei, pp. 591-596. DOI: 10.1142/9789814632041_0065.

Journal article in press:

George Michael Ndilowe; Olga Kristavchuk; Yuri Kochnev; Alexander Nechaev; Leslie Petrik. 2018. Preparation and Characterisation of Silver-coated Polyethylene Terephthalate Membrane" Polymer Bulletin (POBU)

Dr Chris Ademola Bode-Aluko, Dr Katri Laatikainen, Dr Yuri Kochnev, Dr Omoniyi Pereao, Professor Leslie Petrik, Dr Catherine Branger, Professor Alexander Nechaev Removal of Rhodamine 6G from aqueous solution in a continuous mode using novel ultrafast nano-filtration membranes. Submitted to: Separation and Purification TechnologySEPPUR_2018_2820

The Russian counterparts' publications are:

Kristavchuk, O.V. et al. (2017) ‘Immobilization of silver nanoparticles obtained by electric discharge method on a track membrane surface’, Colloid Journal, 79(5), pp. 637-646. doi: 10.1134/S1061933X17050088.

Molokanova, L.G. et al. (2017) ‘Effect of ultraviolet radiation on polyethylene naphthalate films irradiated with high-energy heavy ions’, High Energy Chemistry, 51(3), pp. 197-203. doi: 10.1134/S0018143917030109.

Syrtsova, D.A. et al. (2016) ‘Structure and properties of poly(4-methylpentene-1) track-etched membranes’, Petroleum Chemistry, 56(4), pp. 294-302. doi: 10.1134/S0965544116040113.

Artoshina, O.V. et al. (2016) ‘Structure and phase composition of thin TiO2 films grown on the surface of metallized track-etched polyethylene terephthalate membranes by reactive magnetron sputtering’, Inorganic Materials, 52(9), pp. 945-954. doi: 10.1134/S0020168516080021.

Apel, P.Y. et al. (2015) 'Production of multi-, oligo- and single-pore membranes using a continuous ion beam', Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms. Elsevier B.V., 365, pp. 641-645. doi: 10.1016/j.nimb.2015.07.108.

Artoshina, O.V. et al. (2015) 'Structural and physicochemical properties of titanium dioxide thin films obtained by reactive magnetron sputtering, on the surface of track-etched membranes', Petroleum Chemistry, 55(10), pp. 759-768. doi: 10.1134/S0965544115100011.

Conferences:

L. Petrik. 2018. Educational Programme of the Western Cape University in connection with possibilities offered by Joint Institute of Nuclear Research Russia. 5th South Africa-JINR Symposium. Advances and Challenges in Physics within JINR and South Africa. Nov 4-9, 201. Erinvale, Somerset West. Invited speaker

L. Petrik. 2018. Track etched membrane based nanotechnologies. International School on Nuclear Methods for Environmental and Life Sciences. NMELS'18. April 22-20, Budva, Montenegro. Invited speaker

L. Petrik. 2016. Multifunctional Track Etched Membranes for water treatment and monitoring.10th Collaboration Celebration & Ten-year Review Forum. 28 November to 1 December 2016. CSIR International Convention Centre, Pretoria, South Africa.

D.A. Syrtsova, V.V. Teplyakov, Yu. K. Kochnev, A.N. Nechaev, O.R. Adeniyi, and L. Petrik. 2016. Gas Permeability and Structure of Track-Etched Poly(4-Methylpentene-1). Russian National Conference MEMBRANE-2016 10-14 October, 2016, Nizniy Novgorod, Russia

O. Adeniyi, L. Petrik, A. Nechaev, P. Apel. Swift heavy ion irradiation of polyester and polyolefin polymeric film for gas separation application. (A review on PhD study). 4th SYMPOSIUM in Dubna (21-24 September 2015). Young researcher session

L. Petrik. 2015. Applied Research – new trends in collaboration of SA with JINR. Symposium Few to Many Body Systems: Models, Methods and Applications, JINR Dubna, September 21-24, 2015. 4th SYMPOSIUM in Dubna (21-24 September 2015) Session on Applied Research. Invited speaker

L. Petrik, R. Missengue, P. Du Plessis, V.R.K. Vadapalli, B. Mabova, T. Sonquishe, C. Gilbert, G. Ndayambaje, A. Rossouw, J. Tijani, N. Hintso, V. Somerset, E.O. Omoniyi, W. J. Perold, O. V. Artoshina, A. N. Nechaev, P. Yu. Apel. 2015. Nano for Water Treatment. Emerging Frontiers for sustainable Water. 3-5 Aug, 2015, Johannesburg. Invited speaker.

ACRONYMS & ABBREVIATIONS

APTMS	Aminopropyltrimethoxysilane
CE	Capillary Electrophoresis
DETA	Diethylenetriamine
EDA	Ethylenediamine
EDS	Energy Dispersive Spectroscopy
FTIR	Fourier Transformation Infrared
GC-MS	Gas Chromatography tandem Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
LC-MS	Liquid Chromatography tandem Mass Spectrometry
LOD	Limits of Detection
LOQ	Limits of Quantification
NIR	Near Infra-Red
PCPs	Personal Care Products
PET	Polyethylene Terephthalate
SEM	Scanning Electron Microscopy
SERS	Surface-Enhanced Raman Spectroscopy
SPE	Sample preparation with solid phase extraction
SPP	Surface Plasmon Polariton
TEM	Transmission Electron Microscopy
UV-Vis	Ultra Violet-vis Spectroscopy

1 BACKGROUND

1.1 INTRODUCTION

The identification and detection of persistent and emerging contaminants in waters or wastewaters require reliable, accurate and sensitive analytical techniques with low detection limit, low fouling tendencies and high reproducibility (Fan et al., 2011). Emerging and persistent micropollutants are present in the environment at low concentrations (ng/L to micro g/L). Several analytical techniques such as liquid chromatography tandem mass spectrometry (LC-MS), liquid chromatography tandem mass spectrometry coupled with mass spectrometry (LC-MSMS), high-performance-liquid chromatography (HPLC), high-performance-liquid chromatography coupled with mass spectrometry (HPLC-MS), liquid chromatography coupled with electrochemical detection (LC-ED), capillary electrophoresis (CE) coupled with mass spectrometry, and gas chromatography coupled with mass spectrometry (GC-MS), have been widely used to separate and quantify analyte concentration in various environmental samples (Comerton et al., 2009; Gatidou et al., 2007; Mottaleb et al., 2009). Most of these methods are not without challenges, which include solid-phase extraction and derivatisation of samples in the case of GC-MS prior to analysis. Furthermore, sensor materials are currently considered as a potential substitute to those aforementioned instruments due to the ease of operation and elimination of the pre-treatment steps, lower consumption time, higher sensitivity, and fast responses. Most of the conventional instruments are too cumbersome to operate in the field without skilled personnel to analyse the samples and, despite the long sample pre-treatment, the equipment can only detect or identify a small range of similar contaminants at a time in the matrices. Additionally, most modern facilities lack necessary and selective sensing devices, which limit or affect accurate determination of the pollutant concentration. Thus, there is a need to develop accurate and sensitive analytical protocols that can screen, detect, and quantify different contaminants concurrently. Surface Enhanced Raman spectroscopy (SERS) with a narrow spectral band is considered as a powerful analytical tool for detection of pollutants in a complex mixture (Klutse et al., 2012). However, the performance of SERS as a detection tool depends on the nature of the platform upon which the SERS analysis is undertaken.

The detection and monitoring technique considered in this study comprised a pre-concentration step followed by surface enhanced Raman Spectroscopy (SERS). The pre-concentration step involved utilisation of a supported track-etch membrane containing arrays of silver or gold nanoparticles. In this study, novel surface enhanced Raman Spectroscopy (SERS)-active supports containing arrays of silver or gold nanoparticles on the surface of track-etch polyterephthalate membranes were developed. The nanoparticles were anchored on the porous PET track membranes using a self-assembled monolayer containing thiolated organic molecules. The self-assembled-monolayer modified SERS membranes were used for the detection of selected emerging and persistent contaminants in waters and wastewaters. The pre-concentration steps consisted of (i) rapid separation and up concentration of the trace contaminants from the aqueous matrix, which was achieved by filtration through the small nano

sized pores in the track etched membrane and (ii) improving the detection sensitivity of SERS by the surface enhanced silver plasmon nanostructures to detect organic pollutants at low concentrations. It is envisaged that this new spectroscopic method will be suitable for rapid screening and sensing contaminants in environmental samples that require low detection limits of analytes as well as fingerprinting capability.

1.2 AIMS OF THE PROJECT

The aim of the project is to investigate and develop detection/monitoring methods for a large range of emerging and persistent contaminants. In order to achieve the aim, the following objectives were considered;

- Development of clear, concise and suitable effluent sampling procedures
- Development of suitable extraction procedures for the detection of a variety of emerging micropollutants
- Development of a new rapid screening analytical method based on plasmonic nanosilver coated track-etched membranes that can be used as a platform for Surface Enhanced Raman spectroscopy (SERS) for the detection of emerging micropollutants in water samples
- Determining the effect of different matrices upon detection and quantification of emerging micropollutants
- Determining the limitations of various sampling, extraction and analytical techniques for detecting different categories of emerging micropollutants in water samples
- Comparison of the new analytical method with existing analytical procedures in order to determine the advantage of the new method over the existing ones

LITERATURE REVIEW

1.3 BACKGROUND

The unavailability of fresh and clean water has become of global concern and attracts scientific attention. The non-availability and deterioration of water quality globally can be attributed to natural and anthropogenic activities such as population growth, climate change and rapid industrialisation in conjunction with lack of functional wastewater treatment facilities. Through these processes, many chemicals have found their ways into water bodies thereby compromising the quality of our water systems. These chemicals include pharmaceuticals and veterinary medicines, endocrine disrupting compounds (EDCs), personal care products (PCPs), flame retardants, perfluorinated and brominated substances, pesticides and herbicides, as well as nanomaterials amongst others (Houtman, 2010; Fawell and Ong, 2012). The extensive use of organic compounds in pharmaceuticals, pesticides, personal care products, and other consumer products such as those containing surfactants has increased the presence of these chemicals in the environment. For instance, pharmaceutical and personal health care products include over-the-counter and prescription drugs such as antibiotics, analgesics, blood lipid regulators, natural and synthetic hormones, beta-blockers, anti-diabetics, antihypertensives, etc. The ingredients of soaps and/or detergents, perfumes, skin and hair products and dental care products are part of a diverse group of compounds found contaminating the environment. These and other products that are used in everyday life (Yu et al., 2013, Ternes et al., 2004) have made their way into water bodies. In South Africa, many chemicals of emerging concern have been identified in water and wastewater sources. Patterton (2013) conducted a scoping study on emerging contaminants in drinking water in some cities in South Africa and discovered the presence of over 32 compounds, comprising predominantly pharmaceuticals and pesticides. Such studies have shown that many emerging contaminants are increasingly detected in water sources as a result of industrial processes and disposal of untreated wastewater, with pharmaceuticals and related compounds topping the list. The compounds detected by Patterton in the water samples included atrazine, carbamazepine, cinchonidine and terbutylazine. The presence of these contaminants in water has become life threatening for humans and ecosystems. For example, the veterinary use of diclofenac, which is a human pharmaceutical used as an anti-inflammatory treatment, was found to be responsible for the massive decline in populations of vulture species in certain areas of Asia (Oaks et al., 2004); the veterinary drug ivermectin, which is used to treat parasitic infections in livestock, has been shown to affect the growth of aquatic invertebrates at concentrations lower than those that are expected to occur in the aquatic environment (Garric et al., 2007); ethinylestradiol, one of the active ingredients in the contraceptive pill, has been associated with endocrine disruption in fish (Lange et al., 2001). There is a high concern that long-term exposure to antibiotic pharmaceuticals, used in human and veterinary medicine, may be contributing to the selection of resistant bacteria in the environment, which may have significant implications for human health (Boxall et al., 2004). Therefore, proper identification, monitoring and quantification of these compounds in our waters are needed in order to understand their

presence. Moreover, their chemical properties will influence the choice of effective degradation pathways so as to reduce their effect on human health and ecosystems. However, monitoring of organic pollutants in waters is a difficult task due to the complexity of most of the persistent organic pollutants. For instance, out of thousands of organic pollutants that may have endocrine disrupting effects, methods of extracting and detecting of only a few compounds have been developed. Despite the fact that efforts have been made by researchers in South Africa to detect some of the emerging micropollutants in water systems, only a few cities in the country have been monitored for some compounds. There are several cities in South Africa where no studies have been done relating to emerging micropollutant monitoring, and this limits the available information on all the categories of emerging micropollutants present in South Africa waters that need to be removed or degraded. In order to be able to detect and monitor the persistent and emerging micropollutants with ease, there is a need to develop suitable analytical methods for detecting and screening as well as for identifying these contaminants. A few studies have developed methods to identify various classes of contaminants in water and wastewater treatment plants before and after treatment processes (Swartz et al., 2006). However, in most cases where detection methods have been developed, the developed identification methods only target one or two classes of organic pollutants while several other compounds that may have endocrine disrupting and other health effects are yet to be identified. This is probably due to the complexity of their chemical properties, as well as low concentrations in the environment, the complex matrices in which these compounds occur (Petrovic *et al*., 2003), their high polarity and their thermal stability (Yang *et al*., 2007). Some analytical techniques, including high performance liquid chromatography and gas chromatography coupled with mass spectrometry, which have been used worldwide, could only detect a limited number of organic contaminants in water and wastewater. For accurate monitoring of persistent and emerging contaminants in aqueous environmental samples (ground, surface, drinking and waste water), there is thus a need for the development and validation of precise and highly sensitive analytical techniques that can effectively screen for the presence and also identify and quantify contaminants at very low level (ng/L). This will enable researchers to have a good understanding of the effluent composition before and after treatment. Having a clear knowledge of the nature of contaminants in the effluents before and after treatment is also necessary in the development of treatment methods. The focus of this study is the development of a new platform to separate, identify and quantify persistent and emerging contaminants using surface enhanced Raman Spectroscopy (SERS) as analytical method.

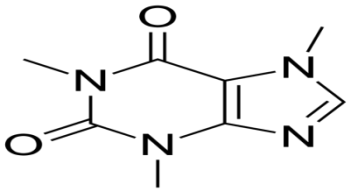
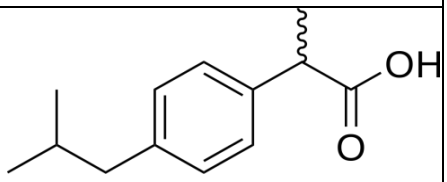
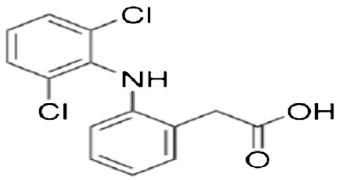
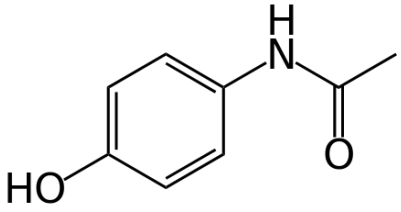
1.4 ANALYTICAL TECHNIQUES FOR DETECTION OF EMERGING AND PERSISTENT CONTAMINANTS

The drugs under study in this report are Caffeine, Diclofenac, Ibuprofen and Acetaminophen, they have been known to pose health risk to aquatic life and pose a danger to the environment due to their persistence (Table 1.1). Caffeine is a natural alkaloid found in foods, beverages and drug materials. It functions as a stimulant in cardiac, cerebral and respiratory systems. It also enhances the effect in certain analgesic drugs (Paiga & Delerue-Matos, 2017). Consequently the global average consumption is between 80 mg and 400 mg per person per day. It has been reported that only 0.5-10% of caffeine

is metabolised by the body for energy with the rest being excreted through urine and faeces. Its characteristic properties of high solubility in water and a half-life of approximately 10 years make it a suitable marker of domestic wastewater (Viviano et al., 2017). Reports of caffeine as a data rich emerging contaminant in environmental matrices dates back to 1978 (Gil et al., 2018).

Diclofenac is an analgesic nonsteroidal anti-inflammatory pharmaceutical compound (NSAID). Biodegradation of diclofenac is limited with removals by wastewater treatment plants of only 21-40% hence its detection in water bodies (Chong et al., 2017). Its presence in trace levels in aquatic environment has resulted in the drug being included in the EU First Watch List for emerging water pollutants (Aziz et al., 2017). Diclofenac exposure has adverse effects on animal and human life due to bioaccumulation in tissues. In rainbow trout cytological changes in the gills, liver and kidneys were observed with long exposures of 5 microg/L of diclofenac (Cunha et al., 2017). In humans diclofenac exposures causes nephropathy, gastrointestinal ulceration and idiosyncratic liver toxicity (Guiloski et al., 2017). Ibuprofen an NSAID is used to relieve pain. Ding et al. (2017) reported a decrease in biomass of eukaryotic algae and accelerated growth of the cyanobacteria due to ibuprofen. Algae is an important component in the aquatic food chain and its decline will result in decrease of fish population. This was observed in a study that showed toxicity of Ibuprofen to green algae *Chlorella vulgaris* with a 96 h-EC50 value of 89.65 mg/L. Paracetamol (acetaminophen) is a pain reliever and a fever reducer. In a study done by Rao et al. (2017) they reported that exposure to paracetamol caused behavioural and physical changes in *Cyprinus. carpio* (fingerings and adults). These changes include endo and exodermal irritation, discoloration of scales, fin rot, liver muscle and brain damage which lead to death. It has been observed that low concentrations of paracetamol causes early death in fingerlings compared to adult fish.

Table 1.1: Physicochemical properties of four micropollutants under study Sources (Kaur et al., 2017, Lindholm et al., 2014 and Zhong et al., 2016)

Pollutant	Molecular mass (g/mol)	pK_a[b]	Water solubility (mg/L)	Function	Structure
Caffeine $C_8H_{10}N_4O_2$	194.2	0.6	21 600	Stimulant	
Ibuprofen $C_{13}H_{18}O_2$	206.3	4.4	21	NSAID	
Diclofenac	318.1	4.0	50 000	NSAID	
Paracetamol $C_8H_9NO_2$	151.165	9.5	No data available (approximately 14 000)	Analgesic	

The screening, detection and identification of persistent and emerging contaminants in waters or wastewaters require reliable, accurate and sensitive separation and analytical techniques with low fouling tendencies, low detection limits, and high reproducibility (Fan *et al*., 2011). Emerging and persistent micropollutants are present in the environment at very low concentrations (ng/L to microg/L). Several analytical techniques such as liquid chromatography tandem mass spectrometry (LC-MS), liquid chromatography tandem mass spectrometry coupled with mass spectrometry (LC-MSMS), high-performance-liquid chromatography (HPLC), high-performance-liquid chromatography coupled with mass spectrometry (HPLC-MS), liquid chromatography coupled with electrochemical detection (LC-ED), capillary electrophoresis (CE) coupled with mass spectrometry, and gas chromatography coupled with mass spectrometry (GC-MS) have been widely used to quantify their concentration in various environmental samples (Comerton *et al*., 2009; Gatidou et al., 2007; Mottaleb et al., 2009). All these techniques couple a separation system (GC, HP or CE) with a detection and quantification system (usually MS or FID).

Gas chromatography (GC) and liquid chromatography (LC) coupled to MS are the two most important techniques used for analyte separation prior to identification and quantification of organic pollutants

such as pharmaceuticals and related compounds in environmental matrices. Due to its versatility, high specificity and selectivity, liquid chromatography coupled with MS has become the most preferred analytical technique used for multi-residual organic pollutants' analysis (Anumol et al., 2013). Nevertheless, GC coupled with detectors such as FID and MS can be effectively applied for volatile and non-polar organic compounds. However, GC-MS is time consuming because it requires an additional derivitisation step, which can also increase the risk of analyte loss (Kolpin et al., 2002) (Table 1.2). GC-MS is used to analyse volatile and semi-volatile compounds. Polar compounds are thermally unstable and hence require derivatisation to compounds that have properties suitable for GC analysis. There are different types of chemical derivatisation methods, which include alkylation, silylation and acylation. Derivatisation improves separation, reduces thermal degradation, increases volatility and also increases sensitivity by adding functional groups that allow for higher detector signal. This is a critical step as it may affect the accuracy of the method hence choice of derivatising agent is necessary. Furthermore, some compounds such as carbamazepine are thermo labile and decompose during GC analysis thereby forming degradation products.

Table 1.2: Examples of derivatising agents that can be used for the compounds in this study Sources (Fatta *et al*., 2007)

Pharmaceuticals	Derivatisation	Solvents(elution)	Detection	LOD(ng/L)
Ibuprofen, Diclofenac	N-Methyl-N(tert-butyldimethylsilyl) trifluoroacetamide MTBSTFA)	Methanol Ethyl Acetate	GCMS	1-10
Paracetamol	MSTFA (N-methyl-N-trimethylsilyl-trifloroacetamide	Methanol Hexane	GCMS	2-4
Caffeine	Methanol/BF_3		GCMS	2.9-8.0

LC-MS is well known for its sensitivity, specificity and selectivity in the analysis of trace compounds. (Fatta et al., 2007; Dams et al., 2003). However LC-MS suffers from matrix effect due to suppression of the electrospray ionisation and thereby results in inaccuracy. LC-MS has been noted to have greatly reduced the analysis time but with accuracy and precision being compromised due to matrix effects (Dams et al*.,* 2003). Matrix effects are attributed to co-eluting residues of the matrix on the target analyte. To eliminate the matrix effect efficient clean-up methods with minimal solvent can be used. In other cases spiking the sample with an internal standard showed an improvement in the accuracy of the results. Simple serial dilution also enhances the signal of analytes by suppressing the matrix effect (Fatta et al*.,* 2007). LC-MS is suitable for analysis of large molecular weights and thermally liable polar compounds not suitable for GC-MS. The analyte ions are mechanically and electrostatically separated from neutral ions. The most common types of ionisation are electron spray ionisation and atmospheric pressure ionisation that can interface between the liquid chromatography and the mass spectrometer.

The use of Atmospheric pressure ionisation (API) has improved the sample polarity and flow rates of the old LC-MS tool. The API is useful in analysing large biomolecules whilst atmospheric pressure chemical ionisation (APCI) is used to analyse nonpolar and polar biomolecules of less than 1500u (Kanda & Glendinning, 2011). Hol-capek et al. (2012) noted that the fastest analyser coupled to LC-MS is the TOF analyser with speeds of 10-50 Hz. LC-MS operating procedure includes separation by the liquid chromatography, ionisation and identification by mass spectroscopy (Table 1.3).

Mass Spectroscopy (MS) is an analytical technique that separates ions according to their mass to charge ratio allowing for measurements of chemical composition and structure. MS has the advantage of increased sensitivity, specificity and selectivity and when coupled to GC and LC improves the data quality. Hao et al. (2007) also highlighted that MS allows for use of isotope labelled compounds to be used which corrects matrix effects, especially for LC-MS. MS often faces challenges such as ion suppression effects, poor discrimination between isobaric compounds, and the requirement for derivatisation of some complex samples (Bell & Sirimuthu, 2008). MS analysis may suffer from signal enhancement or suppression, because of the interference effects of the co-extracted matrix. Consequently, matrix effects have been identified as having major negative effects on LC analysis of pharmaceuticals and other emerging contaminants in environmental matrices (Wille et al., 2012). Hybrid mass analyser like the triple stage quadrupole mass spec allow for tandem mass spec ms/ms and quantitative analysis of low concentration compounds and also for those with a high matrix signal. The analysis that can be performed include product ion scans and selected reaction monitoring (Kanda & Glendinning, 2011).

Table 1.3: Examples of types of mass analysers that can be coupled to LC-MS (Holčapek et al., 2012)

Mass analyser	Instrument name, manufacturer	Resolving power(FWHM defined at m/z)	Resolution ($\triangle$m/z)	m/z range	Acquisition speed (Hz)
TOF	Citius , Leco	100 000, (m/z 609)	0.006	50-2 500	200
Q-TOF	TripleTOF 5600, AB Sciex	35 000,(m/z 956)	0.03	5-40 000	25 MS, 100 MS/MS
Q-ICR	SolariX 15T, BrukerDaltonics	2 500 000, (m/z400)	0.0002	100-10000	-
Q	LC/MS Purification system, Gilson	-	1	50-3 000	10[b]
IT	LCQ Fleet, Thermo Scientific	-	0.3	15-4 000	12[b]

LC-MS/MS allows the determination of a wide range of compounds. According to literature, slightly better limits of detection (LOD) can be achieved with LC-MS/MS compared to GCMS. Kostopoulou and Nikolaou (2008) gave the sample preparation protocols, GC-MS and LC-MS analytical conditions, and

limit of quantitation of the pharmaceuticals that were identified from 1998 to 2008. Methanol and acetonitrile in Milli Q water are used as mobile phase for LC. To achieve satisfactory retention and reproducible retention time for acidic organic compounds, mobile phase acidification or the use of a buffer in the eluent is recommended. Volatile compounds such as formic acid and ammonium acetate are the most desirable mobile phase additives for anti-inflammatory drugs and antiphlogistics. Figure 1.1 shows the process for the identification of organic compounds (pharmaceuticals) using GC-MS and LC-MS methods.

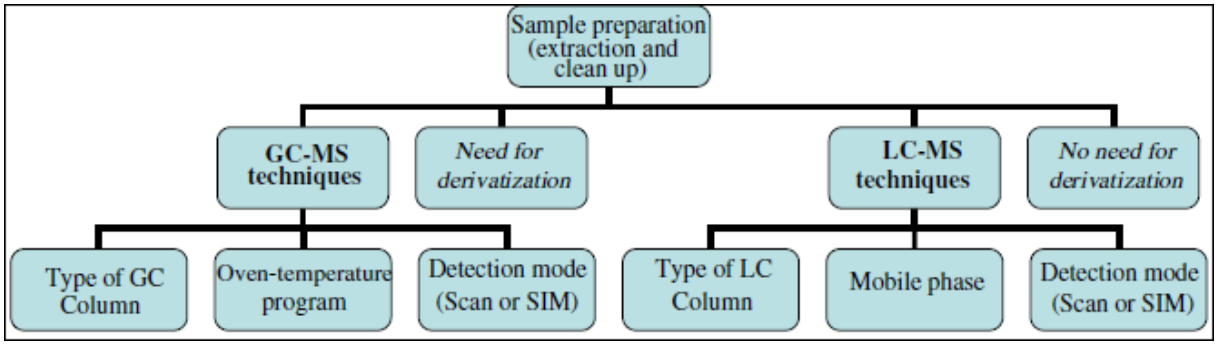


Figure 1.1: Features of GC-MS and LC-MS methods for determination of pharmaceuticals (Kostopoulou and Nikolaou, 2008).

Lately, ultra-high-performance LC (UHPLC), which is capable of operating at very high pressures (>1000 bar) has been developed. UHPLC uses columns with sub-2 µm particle diameter, which allow faster separation of compounds. It also increased resolution sensitivity, and reduced matrix effects more than conventional LC (Jernberg, 2013). Pharmaceuticals can be analysed with different ionisation interfaces namely, electrospray (ES1) and atmospheric pressure chemical ionisation (APCI). An analysis of antimicrobials (six sulfonamide and five tetracyclines) was performed by Lindsey and co-workers (2001) using multiple positive and negative LC-MS ionisation mode under optimised conditions. Both ESI and APCI in the positive modes were reported to be effective but ESI was chosen over APCI because it was more sensitive towards chrotetracycline. Furthermore, the most recent LCMS/MS techniques include triple quadrupole (QqQ), quadrupole-time of flight (QqToF), ion trap (IT), and quadrupole-linear ion trap (QqLIT). QqQ and IT instruments are in common use. They allow the detection of pharmaceuticals at low (ng L^{-1}) level (Perez and Barcelo, 2007). QqTOF analytical instruments have been applied for the elucidation of structures suggested for transformation products (Gomez et al., 2007). Seitz et al. (2006) applied QqLIT methods for the determination of carbamazepine, diclofenac, and iodinated X-ray contrast media in wastewater samples.

1.5 SAMPLE PREPARATION

In sample preparation, filtration is usually suggested before the extraction in order to remove particulate matter from the samples, but rarely for pre-concentration of analytes. Membranes used for micro filtration have pore sizes of approximately 1.0-0.01 microns and can be used to remove substances such as sand, silt, clays, algae and some bacteria. The membranes used for ultrafiltration have pore

sizes of approximately 0.01-0.001 microns and are used to remove microbiological materials, macro molecules and particles. The membranes used for nanofiltration have pore sizes between 0.0001 to 0.001 microns. Pushing water through such small pores requires higher pressure compared to MF and UF. Nano-filtration membranes are capable of removing low molecular weight organic compounds, dyes and divalent ions and, as such, track etched membranes are widely used for rapid analyte separations as is further detailed in Section 2.5.

Washing of the filters with methanol or a suitable solvent after filtration is recommended because some fraction of the target compounds may be removed with the suspended solids during filtration (Vanhaecke et al., 2011). Depending on the sample matrices, the levels of pharmaceuticals in the environment are normally in the ng/L-g/L or pg/g-ng/g range. Extraction and clean-up is important to transfer analytes of interest from complex matrices to a simple solution and for the extract purification, to reduce/exclude interferences that may be co-eluted with the analytes before quantification (Vanhaecke et al., 2011). There are different extraction methods, developed for the extraction of pharmaceuticals from aqueous samples. Hou et al. (2013) reported that various pre-treatment techniques have been developed to extract phenolic compounds from aqueous samples, such as solid-phase extraction, solid-phase microextraction, single-drop microextraction and hollow-fiber liquid-phase microextraction. The abovementioned authors developed a method for dispersive liquid-liquid microextraction of 4-nitrophenol, 2-naphthol and bisphenol A.

Recently, solid phase extraction (SPE) has become the most commonly method of analyte extraction with preconcentration and has replaced the liquid-liquid extraction (LLE) methods for liquid samples (Chang et al., 2011; Anumol et al., 2013). In solid phase extraction, analytes of interest are absorbed and thus concentrated onto a solid phase sorbent out of a dilute dispersion. The sorbent is then washed with a suitable solution to eliminate unwanted compounds co-eluted with the target analytes. SPE adsorption of pharmaceuticals has been improved by developing different polymeric sorbents, which are typically hydrophilic-lipophilic balanced (HLB). Oasis HLB, which is a copolymer of divinylbenzene and vinylpyrrolidone, is presently the most frequently used SPE sorbent for multi-residual pharmaceuticals extraction. A general SPE analytical method for aqueous environmental samples includes the use of octadecyl (C18) silica, polymeric, or hydrophilic-lipophilic balanced (HLB) with either disk or the commonly used cartridges. HLB are reported to give better results at neutral sample pH. When using C18, there is a need for sample pH adjustment, which depends on the basic, neutral and acidic nature of the target analytes. Other copolymer cartridges used include Isolute ENV+, chromobond HR-X and Strata-X (Kostopoulou and Nikolaou, 2008; Jernberg, 2013).

After extraction, analytes are collected from the sorbent by elution with a pure polar solvent, commonly acetonitrile or methanol. SPE has commonly been achieved before separation and detection of pharmaceuticals (off-line SPE). Lately, online SPE has also proven to be an effective extraction technique. On-line SPE is coupled directly with an analysing system (e.g. LC/MS) or may be used as a fully automated system (Wille et al., 2012). Nie *et al*. (2012) used the SPE to detect several typical

endocrine-disrupting chemicals (EDCs), including estrone, 17β-estrone, 17α-ethinylestradiol, estriol, bisphenol and 4-nonyl-phenol from Beijing (China) wastewater. To avoid analyte loss in some case, PTFE bottles are preferable to glass containers. Chelating agents such as Na_2EDTA can also be added before extraction, in the case of antibiotics such as sulphonamides, tetracycline, and polypeptide antibiotics. Molecularly Imprinted Polymers (MIPs) have been effectively used as alternatives to SPE for the extraction of various classes of pharmaceuticals such as beta-blockers, non-steriodal, anti-inflammatory drugs and antidepressants. The level of co-extracted matrix compounds is reduced with MIP technique, because the sorbents are custom-made for specific target analytes (Bravo et al., 2007; Zorita et al., 2008; Dai et al., 2011). Dai *et al*. (2011) used a MIP as solid-phase extraction material for the quantitative enrichment of diclofenac in environmental water samples. Bravo *et al*. (2007) described the synthesis and use of a MIP as sorbent for on-column solid-phase extraction of diethylstilbestrol from aqueous samples. Other techniques used for simultaneous extraction, clean-up and concentration of pharmaceuticals in aqueous samples include liquid-phase microextraction (LPME) and solid-phase microextraction (SPME) (Moeder et al., 2000). Moeder *et al*. (2000) reported the use of SPME method for determining trace amounts of polar, biologically active substances in water systems. These different approaches show that there still is not a single, fully integrated system that conveniently separates, identified and quantifies the many thousands of persistent organic contaminants found in trace quantities in the environment.

1.6 SURFACE-ENHANCED RAMAN SPECTROSCOPY

Surface-enhanced Raman spectroscopy (SERS) is a combination of vibrational spectroscopy and surface chemistry techniques, where the Raman scattering signal enhancement is provided by localised surface plasmon resonance in metallic nanostructures (Le Ru and Etchegoin, 2008). SERS is, therefore, an advanced extension of Raman spectroscopy (Lamsal et al., 2012). SERS application can be done where ordinary Raman spectroscopy is short of an identifiable signal from a chosen analyte. Although the SERS application has extensively been studied in the life sciences sector, it is only now finding its way into the chemical and materials sciences (Schlücker, 2014).

Raman spectroscopy, as an analytical technique, is based on the interaction between molecules and photons that result in scattering of radiation (McMahon, 2008). This kind of spectroscopy is based on the detection of vibrations arising from changes in polarisability of bonds in an excited molecule due to the ‘Raman Effect’. The phenomenon that occurs when the inelastic monochromatic light is scattered by molecules at a different wavelength to that of the incident light is known as the ‘Raman Effect’ (Lombardi and Birke, 2009). This ‘Raman Effect’ occurs when a molecule is struck by electromagnetic radiation resulting in a shift of the wavelength of the inelastically scattered radiation. When the Raman shift is towards longer wavelengths, it is called Stokes scattering and when shifted towards shorter wavelengths, it is termed Anti-Stokes scattering (Meyers, 2000). Rayleigh scattering is when the overall Raman shift is zero, i.e. there is no energy increase or decrease related to vibrational energy levels in the ground electronic state of the molecule (Meheretu et al., 2014). The Raman shift occurrence can

be illustrated in the energy diagram in Figure 1.2. Raman scattering occurs when a laser in the visible or near infrared strikes a molecule and interacts with the electron clouds of that molecule.

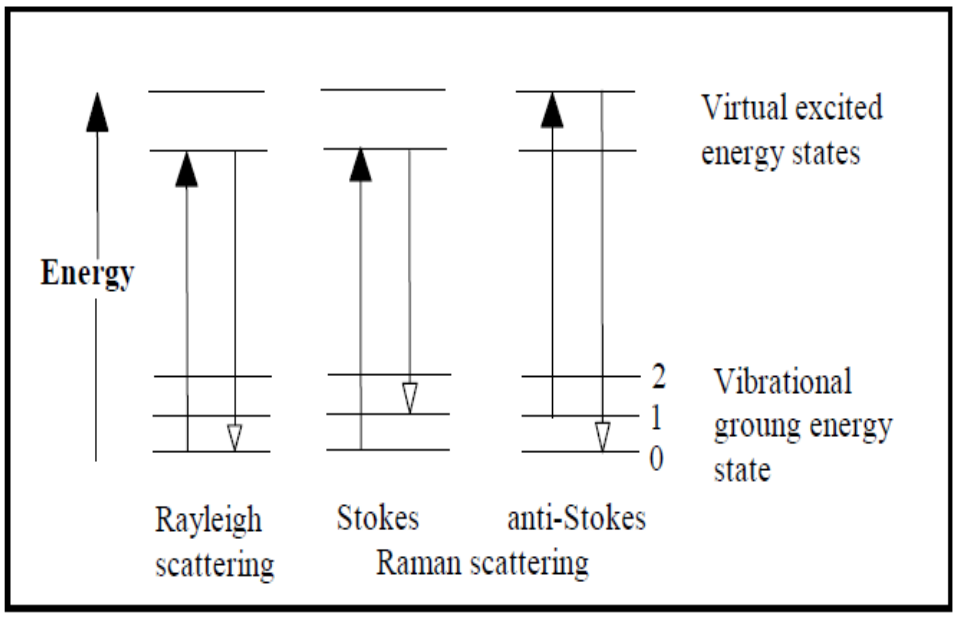


Figure 1.2: Energy level diagram showing the states involved in the Raman spectrum and the elastic and inelastic Raman scatterings (Rayleigh, Stokes and Anti-Stokes).

Raman scattering also depends on the polarisability of molecules (Ferraro, 2003). The incident photon energy in the laser can excite vibrational modes of the molecule to yield scattered light with diminished energy (Reichenbächer and Popp, 2012). Raman signals are relatively weak when compared to infrared signals and they are easily obscured by fluorescence (Boujday et al., 2015). The advantage of Raman over infrared is that it can be used in the aqueous environment since water causes a negligible Raman Effect (McMahon, 2008; Matousek et al., 2006). Raman spectroscopy has not been extensively used due to its weak signals. For this reason, lately interest has been focused on surface enhanced Raman scattering through which the Raman signal is enhanced by metal nanostructures (Le Ru and Etchegoin, 2008).

SERS applications gained a lot of attention due to its sensitivity and ability to detect very low concentrations, and small volumes (Zaleski et al., 2014). SERS relies on electromagnetic and chemical interactions acting on an analyte that has been adsorbed on the surface of a metal support, which has been irradiated by the laser (McNay *et al*., 2011). In SERS, the Raman scattering signal is amplified by the excitation of localised surface plasmons on a roughened metal which generates amplified electromagnetic fields (Sharma *et al*., 2013) and by charge-transfer occurring in the analyte-metal complex (Halvorson and Vikesland, 2010). SERS is Raman scattering that enhances vibrational absorbance of analytes adsorbed on or those in close proximity (Lamsal et al., 2012) to metal nanoparticles or roughened surfaces (Boujday et al., 2015; Craig *et al*., 2013). Plasmonic metallic nanostructures increase the signal intensities of Raman scattering, which enable detection of single molecules (Gühlke *et al*., 2016).

SERS was first observed in 1974 (Boujday et al., 2015) during the study of pyridine on a roughened silver electrode but was not recognised as an enhanced surface Raman scattering phenomenon until it was again reported in 1977 (Sharma et al., 2013). Later, other authors in their studies claimed to have detected a single molecule of the analyte of interest (Kleinman et al., 2013). Over the years the explanation about the occurrence of the SERS signal enhancement has been debated and is resolved to be caused by contributions from the electromagnetic effect, a chemical mechanism, as well as molecular resonance enhancement (Muehlethaler et al., 2015). The electromagnetic effect arises from the excitation of the localised surface plasmons on the nanoparticle metal surface by the electromagnetic wave (Pinzaru et al., 2004; Stiles et al., 2008). This localised field around the surface of the nanometalpolarises the analyte on the surface, and it radiates light, creating a Raman scattering which then interacts with the nanometal structure to produce a new field, which is of the order of 10^{14}. The electromagnetic effect thus arises from the interaction of the excitation laser with an oscillating electron wave on the metal surface (Halvorson and Vikesland, 2010). Electromagnetic enhancement is the most prominent (Péron et al*.,* 2009) of all three contributions as it neither requires the molecules of an analyte under study to be attached to the SERS substrates nor the absorption wavelength of the analyte to be equal or near that of exciting laser (Boujday et al., 2015). The chemical effect arises from a metal electron-mediated resonance Raman via a charge transfer intermediate state and the Raman signal is enhanced by 10^2 (Stiles et al., 2008). The chemical mechanism enhancement occurs when the excitation radiation wavelength is resonant with the analyte-metal complex's charge transfer electronic states (McNay et al., 2011). Molecular resonance enhancement exists when an absorption wavelength of the analyte occurs near the exciting laser wavelength (Muehlethaler et al., 2015; Pieczonka et al., 2008). Thus, the molecular resonance enhancement is rarely cited by earlier authors (Lombardi and Birke, 2009). The immediate decay of the electromagnetic effect ensures that only the adsorbate molecules near or on the noble metal surface are polarised which ensures efficient maximum polarisation of the adsorbate.

The energy from the surface plasmon is then absorbed by an analyte nearby or adsorbed upon the metallic particles on the support, which then transfers the energy back to the support less the amount it transferred to its nucleus (McNay et al., 2011). Surface plasmon resonance (SPR) is the ensemble movement of surface electrons of random or ordered metal nanostructures (Banholzer et al., 2008). Upon excitation of the metal in the charge transfer complex by the incident laser, an electron-hole is created through which energy is transferred to the analyte and Raman scattering occurs (McNay et al., 2011). The magnitude of the enhancement mechanism depends on the morphology of the metal nanoparticles, the localisation of a molecule, the excitation wavelength and light polarisation (Craig et al., 2013; Lombardi & Birke, 2009). The dominating effects of spectral enhancement characteristics of the analyte caused by surface roughness are the surface plasmon resonance of the metallic surfaces and their corresponding variations (Craig et al., 2013; Pieczonka et al., 2008). The collective excitation of surface electrons (surface plasmon resonance) is affected by the distance between metal nanoparticles, the shape of nanoparticles and their dielectric functions (Rodrigues et al., 2013). The

plasmonic metal nanostructures cause resonance Raman scattering that results in enhancement of both incident light and the inelastic light scattered by the adsorbed analyte (Craig et al., 2013).

There is a wide range of applications of SERS as an analytical technique in the fields of biotechnology (Boujday et al., 2015), food industry, warfare anti-terrorism, drug abuse (Craig et al., 2013) and environmental applications (Halvorson and Vikesland, 2010), electrochemistry, surface and material science (Hering et al., 2008). The SERS technique has attracted many researchers in environmental pollutant analysis, with the main focus on screening and detecting, identifying and quantifying very low concentrations of pollutants found either in air or water sources (Le Ru and Etchegion, 2008). The driving force behind research and development of SERS is the trace level analysis capability of the technique and its cost effectiveness (Lucotti and Zerbi, 2007). The technique also offers good practical utility (Li et al., 2014). SERS has several analytical advantages over other methods including ultra-sensitivity, selectivity and inherent molecular specificity (Boujday et al., 2015; Huh *et al*., 2009). Chemical analysis by SERS requires little or no sample preparation (Zhang et al., 2015), is convenient, and is cost effective for development of miniaturised equipment (Ma et al., 2015; Lucotti and Zerbi, 2007). SERS has an edge over infrared spectroscopy, as it can be directly applied in the aquatic environment with negligible background noise due to low polarisability index of water (Li et al., 2014). Although similar techniques such as fluorescence are already well established, the emerging SERS has attractive properties such that it can be used both in the near-infrared and the visible spectral region and does not require labelling the analyte of interest as is practised in the fluorescence technique (Cialla et al., 2012). The performance of SERS is based on its sensitivity, which depends on the surface properties of SERS-active substrates (Botti et al., 2014) that can be tailored to suit the intended application (Péron et al., 2009; Costa et al., 2006). It is envisioned that SERS could be used to detect multiple pollutants in a sample reliably, rapidly and at a lesser cost (Halvorson and Vikesland, 2010). The SERS technique can be used to identify unique molecular signatures in a matrix of the analytes that have similar structures (Bantz and Haynes, 2009). The SERS technique is flexible such that it can be applied in sequence with other separation techniques, such as nanofiltration polymer membrane technology or chromatography (Muehlethaler et al., 2015), scanning probe microscopy and microfluidics (Cialla et al., 2012). SERS can be coupled with separation techniques such as liquid chromatography and capillary zone electrophoresis to counteract the problem of band overlapping (Subaihi et al., 2017). Quantitative analysis is achieved by optimising the enhancing media and the experimental conditions. Optimising the experimental conditions can be achieved by use of a standardised flow injection and microfluid systems such as polydimethylsiloxane which reduces mechanical and optical variations. However, with flow systems internal standards are used which eliminate laser power variations and correct minor optical variations. The internal standard used should be a non-SERS-active internal standard such acetonitrile, that does not interfere with the analyte signal and is detectable in a non-interfering spectral region (Bell and Sirimuthu, 2008). Table 1.4 gives a summary of a SERS instrument.

Table 1.4: Components of a SERS Instrument, Sources (Halvason et al., 2010)

Component	**Function**	**Choices**
Laser	Provides a beam of light of a certain wavelength that can excite the nanometallic structures on the substrate	Visible to Near Infrared region can be utilised
Analyte	Polarisable analyte produces a Raman signal as it vibrates in its ground state	Solid, liquid, slurries
Enhanced surface	Produced an electromagnetic field which polarises the analyte. Then also enhances the Raman signal produced by the analyte	-fabricated nanometric metallic patterns on solid supports - films doped with nanometallic structures such as Au, Ag
Detector	Records peak intensity against Raman shift due to the vibrations of the bonds in the molecule. These can be used for identification and quantitative analysis of pure compounds.	Intensified CCDs, NIR detectors,

Emerging pollutants exist in the nano range and at very low concentrations thus limiting the sensitivity of GC-MS and LC-MS instruments. Pharmaceutical contaminants are normally present in a matrix and as a result the matrix effect impact the quality of the results or the detection of some drugs. These xenobiotics which ought to be removed from water sources in water treatment plants cannot be detected by normal analytical tools and thus exist in the final water source which is tap water. Furthermore GC-MS and LC-MS methods require large volumes for analysis whereas the new SERS technique only requires a droplet on the surface, facilitating analysis of very small volumes. In addition the traditional techniques employ expensive solvents in large quantities to run making the whole analysis expensive whilst initiating an environmental hazard. A novel method, SERS, was therefore developed to overcome this problem of sensitivity and hence increase detection of pharmaceutical compounds even at very low concentrations. A comparison of the techniques is shown from literature in Table 1.5.

Table 1.5: Summary comparison of the LC-MS, GC-MS and SERS from literature (Megson et al., 2016, Zachhuber et al., 2012, Halvason et al., 2010, Hol-capek et al., 2012*)*

INSTRUMENT	**LC-MS**	**GC-MS**	**SERS**
Sample analytes	Inorganic, organic molecules, biomolecules, ions	Inorganic, organic molecules, Must be volatile	Inorganic, organic, ionic
Sample phase	Liquid	Gas(semi-volatile and volatile)	Solid, liquid, slurries
Sample volume	1 ml	0.5 uL	Droplets
Temperature Pressure	No temperature Pressure	Oven temperature	No temperature Not required
Sample mobility method	High pressure pump with liquid	Carrier gas(N_2 , Ar, He)	Not required
Machine cost	Expensive	Cheap	Very cheap
Maintenance	High	High	Little maintenance
Detectors	Photodiode array, UV/VIS, MS	Thermal conductivity detector(TCD), Flame Ionisation (FID), Electron Capture (ECD), MS	NIR detectors, Intensified CCD detectors
Method of separation	Via liquid chromatography	Via gas chromatography	Does not have, may use LC
Analysis time	Quicker analysis	Slow	Real time response
Sample preparation Solvents	Simple Large volumes of solvents required	Extensive Large volumes of solvents required	Little or not required Small or none are used
Column Cartridges	Short and wide Packed column Require cartridges	Long and narrow Packed/Capillary column Require cartridges	Not required Not required
Destructive	Yes	Yes	None
Application	Separation, identification and quantification	Separation, identification and quantification	Identification and quantification

Farre et al. (2007) analysed pharmaceutical compounds in water using Merck LiChrolut EN cartridges for SPE, while the separation process was achieved using a C18 column LiChrospher 100 RP-18 followed by a guard column (434 mm, 5 mm) with the same packing material. The detection was performed using a HP 1040 M diode array UV-Vis detector coupled in series with the LC-MSD HP 1100 mass selective detector which further was attached to an ESI interface. The results of their analysis showed that 43 ng/L and 5 ng/L (LOD) of ibuprofen and diclofenac sodium respectively were detected. The authors further proved that the derivatisation and concentration processing with GC-MS was time consuming and hence making LC-MS the adequate analytical method in the research work.

In addition Gros, Petrovic and Barcel (2006) conducted an LC-MS/MS similar work, whereby ibuprofen, diclofenac and acetaminophen compounds were identified using polymeric Oasis HLB for SPE. Their objective was to demonstrate that simultaneous extraction of 29 multiple class pharmaceuticals in a single extraction step was possible thereby simplifying the sample preparation step. Interestingly the use of MRM mode, with two transitions examined for each compound, provided good sensitivity and selectivity of detection according to the EU Commission Decision 2002/657/EC. The chromatographic separation was performed using HPLC system coupled to a Waters Micromass Quattro triple quadrupole mass spectrometer coupled to a Z-spray ESI interface. They obtained LOD of 60 pg, 42 pg, 29 pg for ibuprofen, diclofenac and acetaminophen respectively for surface water and waste water treatment plant effluent WWTP. However, they faced challenges with ibuprofen, gemfibrozil and pravastatin because of their low fragmentation but proved that the method was robust and reliable.

Apart from these Lacey et al. (2008) identified ibuprofen, diclofenac and caffeine from influent and effluent water using reverse phase chromatography as it was applicable to many compounds such as pharmaceutical compounds. The authors used Strata-X cartridges (200 mg/6 mL) for SPE preceded by LC-ESI-MS/MS analysis using an Agilent1100 LC system coupled to a BrukerDaltonicsEsquire-LC ion trap MS with an ESI. The capillary column for separation used was A Waters Sunfire, narrow bore, 150 mm×2.1 mm C18 column with 3.5 um particle size. In their experiments, they found the LOD for diclofenac, ibuprofen, and caffeine in influent water to be 0.855, 0.228, 0.280 respectively whilst for the effluent was it was 0.743 for diclofenac, 0.138 ug/l for caffeine. Unfortunately they could not detect ibuprofen hence they could not attest that the method was suitable for such POPs.

In 2002, Miao and others were motivated to develop a method to analyse acidic drugs in aqueous media using LC-MS-MS. In the process LiChrolut 100 RP18 (40-63um) solid phase material manually packed into polypropylene cartridges were used for SPE. This was followed by a chromatographic separation using Waters 2690 HPLC furnished with a Genesis C18 column. The Micromass Quattro LC triple quadrupole mass spectroscopy with a Zspray-electronspray interface in a negative ion mode was employed for mass to charge separation of the ions, which were hence identified. These authors obtained LOD of 10 and 5 ng/L for diclofenac and ibuprofen respectively. They concluded that the method reduced contamination with good chromatographic resolution and acceptable recoveries for environmental compounds

Furthermore Bueno et al. (2011) developed a solvent free method for the analysis of abusive drugs and their metabolites in environmental water. Direct injection of the samples into the Agilent 1200 HPLC system, column-reversed-phase C18 of 150 mm×4.6 mm and 5 um particle size with a binary pump was used. This was coupled to a hybrid triple quadrupole/Linear Ion Trap mass spectrometer system (5500 QTRAP® LC/MS/MS, AB Sciex Instruments with an ESI operating in the positive ionisation mode. The experimental LOD obtained for caffeine was 200 in influent and effluent water and 100 ng/L for surface water. This direct injection proved to reduce sample preparation time and solvents that are employed in SPE hence a greener approach was attained. Also, the methodology resulted in a reduced matrix effect which afforded an increased robustness of the analysis. They discovered that the IDA method, which combines SRM and two analyses in full scan production mode (EPI) within the same chromatographic run, allowed higher sensitivities to be obtained, though it showed inconstant background noise.

Vanderford et al. (2003) reported development of a protocol that is applicable to environmental matrices by employing SPE and LC-MS analysis. An Agilent G1312A liquid chromatography attached to a binary pump and an HTC-PAL auto-sampler were used. The capillary column used was a 250_4.6 mm Synergi Max-RP C12 column with a 4-ím particle size. A mass spectrometer API 4000 triple quadrupole mass spectrometer was used to identify the ions. In this analysis both ESI positive/negative and APCI positive/negative modes were used to fragment the ions. They obtained LOD of 0.3 for acetaminophen, and 0.28 for caffeine, 0.32 for Ibuprofen and 0.12 pg for Diclofenac, in the ESI positive ion mode. In the analysis the recoveries of acetaminophen were enhanced by using concentrated sulphuric acid as a preservative agent preventing the degradation at a pH of 2. The authors concluded that enhancement of the recoveries of various pharmaceutical drugs can be achieved by using concentrated H_2SO_4 to prevent degradation.

To achieve analysis with GC-MS Gumbi et al. (2017) analysed 8 pharmaceutical compounds in river sediments. The research involved ultrasonic treatment and centrifugation and use of Oasis HLB 6 cc 60 mg LP for SPE followed by a derivatisation process whereby 50 µL of a mixture of BSTFA +1% TMCS were added to the samples. Separation was achieved using a GC-MS (QP2010SE Shimadzu) equipped with an auto injector (AOC-20i) and an auto sampler (AOC-20s). The capillary column used was a (intercap SMS/Sil 0.25 mL) with a column temperature of 700˚C whilst the injector pot temperature was kept at 250˚C.The mass analyser used was an ion trap detector ITD operating in the selected ion mode SIM. The LOD for ibuprofen, acetaminophen, diclofenac was in the range of 0.0024-0.443, 0.017-1.595 and 0.092-1.973 ng/g respectively in sand, sediment and biosolids hence the method was suitable for analysis of xenobiotics in environmental solid samples.

In addition Zhong et al. (2016) performed a similar analysis using methylene chloride for the extraction of the pharmaceuticals from sludge. The separation was carried out using GC-MS/MS system (Agilent 7000C) and a scan method for identification. MRM mode was specifically used to determine the pharmaceutical drugs in question. The experimental results for Ibuprofen were obtained at a range of

3.35-4.04 ppm, Diclofenac 0.025 ppm, 0.88-2.11 ppm, Acetaminophen 2.31-3.47 ppm thereby showing the ability of GC-MS/MS to quantify and identify pharmaceutical compounds. Challenges were encountered because of the presence of organic compounds, which affected the ease of the analytical analysis.

In 2008, Togola and others developed analytical procedure to measure a wide range of pharmaceutical compounds in aqueous media thereby improving the sensitivity and reliability of the GC-MS method using an off-line SPE using MCX cartridges. Firstly, samples were filtered on GFF fibre filters and then extracted. Derivatisation was achieved by adding 30 ul of MSTFA to each pharmaceutical drug and a GC-MS analyses carried out using an HP 6890 gas chromatograph from Agilent Technologies with a capillary column called an HP5/MS (30 m×0.25 mm×0.25 m film thickness; phase: 5% diphenyl, 95% dimethylsiloxane). LOD for tap, surface, marine and effluent water were 1.5, 2.5, 2.3, 28.6 ng/L for caffeine, 0.9, 0.7, 2.6, 9.0 ng/L for diclofenac and 0.1, 0.1, 1.7, 4.8 ng/L for ibuprofen. In their study they concluded that using MCX cartridges for SPE resulted in quick, semi-automatic, reliable and reproducible results.

In the case of SERS, literature shows that Zachhuber et al. (2011) reported the quantification of DNT isomers by liquid chromatography with online SERS detection. In the study, LC was used to separate the isomers for SERS analysis. Using a micro-dispenser, samples were deposited onto the enhanced surface and analysed by Raman spectrometry. The authors were able to quantify and identify the isomers due to the structural information obtained from the spectra. They established that quantification method is plausible if SERS is coupled to LC to produce a robust separation method. Interestingly they concluded that the use of the PLS regression assisted in resolving the two isomers and hence they were able to avoid the chromatographic method due to the high signals produced by Raman.

Furthermore Morelli et al. (2017) was able to quantify p-coumaric acid produced by *Escherichia coli* using SERS. In the conducted study, performing liquid-liquid extraction (LLE) was of great importance as this increased sensing in complex medium. Doping the wafer substrate assisted in providing stable and reproducible signals for analysis of complex medium. In addition, in 2013 Guerrini and others managed to quantify oncogenic proteins in cellular extracts by making use of a nanostructured silver surface. The relationship between the marker bands and the concentration allowed for quantification and identification of the oncogene protein c-Jun in complex medium. The authors validated the analytical tool as applicable in diagnosis and chemical biology.

Recently, Zaleski et al. (2017) conducted a study were they aimed at quantifying and identifying intravenous therapeutic drugs using electrochemical SERS and normal Raman Spectrometry (NRS). They successfully detected and quantified gentamicin within its clinically relevant range using both a standard macro and handheld Raman instrument. For drugs that were not measurable NRS EC-SERS was used as a counter to enhance detection. This was successful for dobutamine which was deposited on a Au SERS substrate for analysis. In the analysis they obtained LOD of 10 ng/mL with good accuracy

for dobutamine. They demonstrated that the EC-SERS approach was applicable to secondary amines at acidic pH values. They concluded that this method was adequate for monitoring drug concentrations in clinical settings.

However, analysis of pure standard samples obtained from Sigma Aldrich would eliminate the matrix effect and as a result SPE or LLE can be omitted in this study. Hence, the research approach will include preparation of pure samples to simulate LC and then analyse using SERS

1.7 TRACK ETCHED POLYMERS

Techniques that alter polymer membrane surface properties (surface chemistry) have attracted the attention of researchers in polymer science with the aim of functionalising and immobilising compounds of interest on their surfaces for various applications (Talbert et al., 2012). This is because polymers are inert and lack reactive functional groups on which chemical linkers, metal nanoparticles and biomolecules could be attached (Goddard and Hotchkiss, 2007). This therefore limits their use as membrane structures. As a result, most polymer surfaces required pre or post surface modification procedures in order to achieve the desired properties while maintaining bulk polymer properties (Reznickova et al., 2011; Fatiyants et al., 2013). Polymer membrane surfaces are modified in principle by two methods, chemical methods that involve treatment with reagents and physicochemical methods that use external factors to induce chemical transformations (Fatiyants et al., 2013). The widely used physicochemical surface modification methods are plasma treatment, corona discharge, UV irradiation, surface graft polymerisation and chemical treatment (Dauginet et al., 2001; Marchand-Brynaert et al., 1995). One of the advantages of using physicochemical modification methods is the ability to alter chemical identities such as chemical bonds cleavage, ablation of polymer surface layer, creation of free radicals and conjugation of double bonds of treated polymer (Svorcik *et al*., 2011). There are other means of modifying the surface, which also affect the bulk materials such as the track etching process. Track etched membranes are prepared by bombarding polymer membranes with swift heavy ions (SHI) such as xenon that lead to the formation of ion tracks in the membrane that can be further modified by controlled chemical etching.

Track-etched polyethylene terephthalate (PET) is one the most investigated polymer films as it enjoys the status of being used as a track detector (Apel et al., 1997; Acharya et al., 2006). The various studies on PET under several swift heavy ion and chemical etching conditions (i.e. track-etching) directly relates to the choice of modification in the chemical, thermal, mechanical or physical properties of PET polymer. For instance, it is possible to estimate the percentage loss of atomic composition in polymers such as PET. In understanding polymer interaction with swift heavy ions, the composition of PET film was investigated as a function of ion fluence. Abdesselam et al. (2008) and Abdesselam et al. (2009) reported the preferential release of volatile species especially for molecules that contain hydrogen, carbon and oxygen (Acharya et al., 2006). Also, the order of atomic species depletion observed in PET as a function of ion fluence can be attributed to restricting PET modification to surface properties rather than bulk properties. In a related study by Abdesselam et al. (2011), the percentage loss of hydrogen,

oxygen and carbon during irradiation was reported as 45%, 15% and 85% respectively. For instance, Awasthi et al. (2010) explained the three (3) categories of compounds formed in heavy ion irradiated polycarbonate polymer film, a polyester polymer. These compounds are phenoxy-phenyl, phenoxy-phenoxy radicals and monoatomic species such as H, O and H_2O. The three categories involve intermolecular radical combination. Each of these compounds result in chain scission and decrease in molecular weight as well as branching centres leading to cross-linking. Also, irradiated PET experienced significant chemical modification with the emergence of carbonyl and hydroxyl groups. PET structural alteration after swift heavy ion irradiation in a study by Awasthi et al*.,* 2010 showed the increase in disorderliness including the crystalline identity of PET polymer. The study revealed that surface roughness (morphology) of track-etched PET increased due to an increase in ion fluence.

After ion bombardment to form the latent tracks, the SHI bombarded membranes should then be exposed to selected chemicals such as sodium hydroxide, acids or surfactants to develop the randomly distributed tracks into pores of precise profiles, i.e. shape, size, distribution, etc. in a process called chemical etching (Apel et al., 2015). The pore density on the surface and size of pores are controlled by the density of heavy ions used to create tracks in the membrane as well as the concentration of etching reagents (Dauginet et al., 2001). In the case of other classes of polymers, the use of SHI and chemical etchants to alter physico-chemical properties have been reported. These polymers include polycarbonate, polysulphone, polyimide and polypropylene (Apel et al*.,* 2006, Kumar and Chakarvarti, 2006, Singh *et al*., 2006, Qureshi, *et al*., 2007, Kumar *et al*., 2012).

1.8 MODIFICATION OF POLYMER MEMBRANE SURFACE

Techniques that alter polymer membrane surface properties (surface chemistry) have attracted the attention of researchers in polymer science with the aim of functionalising and immobilising compounds of interest on the polymer membrane surfaces, which are modified in principle by two methods, the chemical methods that involve treatment with reagents and physical methods.

Polymeric membranes are organic membranes synthesised from chemically reactive monomers, which have desirable gaseous and aqueous separation capabilities. Membrane separation processes have been extensively utilised in the aquatic environment due to their benefits, which include the production of high water quality with ease of maintenance, inertness, flexibility and excellent separation efficiency (Lee et al., 2016; Fatiyants et al., 2013; Velleman et al., 2012). Amongst high-performance polymer membranes such as polycarbonate (PC), polyimide (PI), etc., the polymer polyethene terephthalate (PET) has been found to have good mechanical strength, with thermal and chemical resistance (Korolkov et al., 2015; Muthuvijayan et al., 2009). PET is a linear, aromatic polyester type of organic polymer (Marchand-Brynaert et al., 1995). PET, being an inert polymer, lacks the functional groups on the surface to sustainably support SERS-active metals. As such, it necessitates modification (Muthuvijayan et al., 2009). There are several surface modification methods such as plasma treatment,

corona discharge, UV irradiation, surface graft polymerisation and chemical treatment (Dauginet et al., 2001; Marchand-Brynaert et al., 1995).

Track-etched membranes are thin polymer films formed by irradiation with energetic ions of inert gases followed by physicochemical treatment (Fatiyants et al., 2013). The use of track-etched membranes as filters and stable supports for SERS substrates have been studied from different perspectives (Taurozzi and Tarabara, 2007). There are various means of modifying the surface, which also affect the bulk materials such as track etching process. Track etched membrane are prepared by bombarding the polymer membrane with heavy ions, that lead to the formation of pores in the membrane. The membrane is then exposed to selected chemicals such as sodium hydroxide, acids and surfactants to reveal randomly distributed pores of precise profiles, i.e. shape, size, distribution, etc. The pore density on the surface and the size of pores are controlled by density of heavy ions as well as the concentration of etching reagents used to create tracks in the membrane (Dauginet et al., 2001). In this study pre-etched trackmembranes with suitable porosity were further modified as detailed below in order to decorate the surfaces with plasmon metals.

Among the physicochemical modification methods, the plasma process is preferred since it can result in specific reactive species needed for the intended application. For example, oxygen is used in plasma treatment to introduce hydroxyl groups and ammonia for amine groups (Long et al., 2006). The physicochemical methods are considered as precursors to chemical modification when further modification through functionalisation is to be undertaken. The chemical modification techniques are cost efficient and easily used to control both the functionalisation of molecules and immobilisation of nanoparticles respectively (Xue and Lu, 2013). For instance, polymer modification by plasma activation technique could be followed by aminolysis or silanisation (Goddard and Hotchkiss, 2007). However, the most effective way of modifying the surface of the polymer membrane is using wet chemical treatment when all conditions have been optimised to give the best results (Ozcam et al., 2009).

1.9 POLYMER SURFACE MODIFICATION BY CHEMICAL MEANS

The application for which the surface modified polymer membrane would be used often determines the technique of polymer surface modification (Favaro et al., 2007; Park et al., 2013). The wet chemistry parameters such as temperature, time, solvent and concentration must be systematically controlled to maintain consistent surface modification (Drobota et al., 2013; Xue and Lu, 2013). The available experimental data, results and studies has shown that the choice of the chemical linker to be used on a polymer depends on the chemical properties of the compound to be immobilised (Sperling and Parak, 2010). For instance, thiols and amines have a high affinity for noble metal nanoparticles (Braun et al., 2009). Additionally, linker molecules functionalised on surface modified membranes by extension alter the chemical properties of the polymer surface, i.e. aliphatic and aromatic linkers provide a hydrophilic environment (Kubackova *et al*., 2014). The functionalised molecules are usually bi-functional so that one terminal group is chemisorbed to a metal nanoparticle or biomolecule and the other functional group

is coupled to the modified polymer surface (Goddard and Hotchkiss, 2007). However, the amount of immobilised metal nanoparticles or biomolecules is determined by affinity of functional groups such as thiols and amines. This is because noble metal nanoparticles are known to chemisorb on thiols and amine terminal groups resulting in the formation of covalent bonds (Sterling and Parak, 2010). Molecules with poly-functional groups have been coupled to modified polymer surfaces in order to increase reactive sites on the polymer surface for immobilisation of metal nanoparticles and biomolecules (Goddard and Hotcchkiss, 2007).

The organic synthesis route provides the most stable covalent bond (linkage) to immobilise active compounds such as biomolecules and nanoparticles to functionalise polymer surfaces (Goddard and Hotchkiss, 2007). The choice of the chemical linker, therefore, depends on the chemical properties of the compound to be immobilised (Sperling and Parak, 2010). For instance, thiols and amines have a high affinity for noble metal nanoparticles (Braun et al., 2009). In order to functionalise amino acids on a polymer surface, selected wet chemistry conditions that provide for formation of stable amide bonds are employed in cases where the reactive groups on the surface are carboxyl, which are to be coupled with amine functional groups (Deldime et al., 1995). In addition, it is important to optimise the quantity of molecules or nanoparticles to be attached to polymer reactive functional groups (Goddard and Hotchkiss, 2007). Chemical modification through wet chemistry methods has been studied before (Fan et al., 2011; Muthuviyajani et al., 2009). Chemical modifications such as aminolysis, hydrolysis, amidation and carboxylation have been used to introduce reactive functional groups on the surface of polyethylene terephthalate (PET) (Muthuviyajani et al., 2009).The surfaces of PET membranes have been modified using wet chemical treatment by hydrolysis, reduction, aminolysis, carboxylation and glycolysis (Muthuvijayan et al., 2009; Ozcam et al., 2009). This functionalisation is achieved through liquid-solid phase organic synthesis.

Irena *et al*. (2009) in their study on PET surface modification reported that aminolysis of PET depended on choice of amine and treatment parameters such as temperature, time and amine solution concentration. It was shown that the amount of reactive amine functionalities on the PET surface increased with an increase in amine concentration up to a threshold but decreased thereafter even with a further increase in concentration (Irena et al., 2009). In an aminolysis reaction, amine nitrogen through its lone pair attacks an electron deficient carbonyl carbon of the ester link of PET to form an amide bond (Drobota et al., 2013). In a review paper by Goddard and Hotchkiss (2007), it was reported that covalent bond immobilisations provided the most stable bond between functionalised polymer surface and adsorbed compounds (molecules or nanoparticles). In another study by Xue and Lu (2013), comparison of solvents for amine reagents were investigated and the results showed that solvents such as ethanol and dimethyl sulphoxide were more effective than water especially in the introducing of reactive amine functionalities on PET surface. Taurozi and Tarabara (2007) modified polycarbonate with aminosilane via coupling of carbonyl of polycarbonate and amino group of aminosilane forming an amide bond.

1.10 SILVER NANOPARTICLES AND THEIR IMMOBILISATION ON SURFACE MODIFIED POLYMER MEMBRANE

Silver is a naturally occurring metal, appearing most often as a mineral. On the periodic table, silver is positioned as the 47th element and has an atomic weight of 107.8 and has two natural isotopes Ag 106.90 and Ag 108.90 with abundance of 52 and 48% respectively. This metal has been used in a large variety of applications, since it has some unique properties such as high electric and thermal conductivity (Nordberg and Gerhardsson, 1988). Ancient civilizations have used this metal in medicine, food containers and plates, eating utensils, cups, money, jewellery, clothes and water disinfection. The well-known forms of silver are metallic, silver complexes, silver salts and colloidal silver. However, metallic silver dissolves in acids and forms compounds like silver nitrate (Panyala et al., 2008). Aqueous solutions of silver nitrate comprise silver in the form of hydrated silver cations. The silver cation form can be complexed with different organic ligands and even if this cation is still present in the molecule, the charge of the complex can be neutral. Additionally, highly stable complexed forms of silver do not dissociate in solutions or liquids (Panyala et al., 2008).

Nanoparticles are particles that have at least one dimension with a size ranging between 1 to 100 nm. They can exist in single, aggregated or agglomerated, fused forms with different shapes such as spherical, tubular and irregular shape. The common types of nanoparticles are dendrimers, nanotubes, quantum dots and fullerenes. Products with engineered nanoparticles are already on the market and others have been there for several years or decades. These nanoparticles have been used for the creation of new types of analytical tools for life sciences and biotechnology (Cui et al., 2001). In addition, they are also used for ultra-sensitive molecular sensing, diagnostic imaging, photodynamic agents, wound dressings, dental-bonding agents, clothing and electronics. AgNPs are silver nanoparticles with a size smaller than 100 nm and consist of clusters of about 20 to 15,000 silver atoms. Nanoparticles, particularly silver, have various shapes such as spheres, rods and cubes. Furthermore, these particles can be produced as tubes, multifacets, wires and cubes. At the nano-scale the AgNPs have physiochemical properties (such as pH-independence) and greater biological activities compared to the regular bulk metal (Wijnhoven et al., 2009). This is caused by the higher surface area per mass, permitting a larger amount of surface atoms to interact with the surroundings. Nowadays AgNPs have been used in an increasing number of consumer and medical products (Table 2.1) (Panyala et al., 2008). AgNPs in the field of nanotechnology have gained interest because of their unique properties such as chemical stability, good conductivity, antifungal, anti-viral, catalytic, anti-inflammatory and antimicrobial activity. They have been shown to be more effective in preventing bacterial infection and retarding infection in medical based products ranging from topical ointments and bandages for wound healing to coated stents (Chen et al., 2007). In addition these nanoparticles have biological properties which are important for consumer products, textiles/fabrics, food technology and medical applications. Moreover, these nanoparticles have unique optical and physical properties which are claimed to have great potential for medical applications such as drug delivery, diagnostic and imaging (Wijnhoven et al., 2009). AgNPs are now being put in table tops, cutting boards, surface disinfectants and refrigerators in

order to protect humans from food poisoning. Silver nanoparticles can thus be presented in various forms. Table 1.6 lists a variety of applications for silver nanoparticle products.

Table 1.6: Product categories with examples of products containing silver nanoparticles (The values between the brackets indicate the number of sub-categories)

Categories	Subcategories	Examples
Personal care and cosmetics (30)	Skin care (14)	(Body) cream, hand sanitizer, hair care products, beauty soap, face masks
	Oral hygiene (6)	Tooth brush, teeth cleaner, toothpaste
	Hair care (3)	Hair brush, hair masks
	Cleaning (2)	Elimination wipes and spray
	Coating (2)	Make-up instrument, watch chain
	Baby care (2)	Pacifier, teeth developer
	Over the counter health products (1)	Foam condom
Textile and shoes (34)	Clothing (28)	Fabrics and fibers, socks, shirts, caps, jackets, gloves, underwear
	Other textiles (2)	Sheets, towels, shoe care, sleeves and braces
	Toys	Plush toys
Electronics (29)	Personal care (13)	Hair dryers, wavers, ions, shavers
	Household appliances (8)	Refrigerators, washing machines
	Computer hardware (6)	Notebooks, (laser) mouse, keyboards
	Mobile devices (2)	Mobile phones
Household products/home improvement (19)	Cleaning (9)	Cleaning products for bathroom, kitchen, toilets, detergents, fabric softener
	Coating (4)	Sprays, paint supplements
	Furnishing (3)	Pillows
	Furnishing/coating (3)	Showerheads, locks, water taps
Filtration, purification, neutralization, sanitization (13)	Filtration (8)	Air filters, ionic sticks
	Cleaning (6)	Disinfectant and aerosol sprays

The toxic effect of metals is defined as any functional or morphological change in the body caused by consumption, injection, inhalation or absorbed drug, chemical or biological agent. When using either silver metal or silver ions in a reasonable amount, silver has no effect on the human body. Naturally, silver possesses an antimicrobial activity towards many pathogens including bacteria (Zhang and Sun, 2007), fungi, yeasts and viruses. The silver salt (e.g. silver nitrate) has been used to treat mental illness, gastroenteritis, nicotine addiction and infectious diseases such as gonorrhoea and syphilis (Gulbranson et al., 2000). Recently silver coated catheters have been used to stop infections (Samuel and Guggenbichler, 2004). However, ecologists have warned that the widespread use of this powerful antimicrobial agent could have a negative impact for bacteria in natural ecosystems if released in waste streams. There is also growing evidence that silver metal is toxic to bacteria while AgNPs are also highly toxic to mammalian cells (Braydich-Stolle et al., 2005). In addition, these AgNPs have been shown to damage liver, brain and stem cells in animal models. The over-exposure of colloidal or silver salt deposits under the skin causes a skin disease called argyria or argyrosis. Argyrosis is described as a pathologic bluish-black pigment in a tissue which resulted from the deposition of an insoluble albuminate of silver (Panyala et al., 2008). Factors such as solubility and binding specificity to a biological site influence the toxicity of silver metal.

The synthetic route called photochemistry is a chemical reaction that uses light to initiate the transformation (Hoffmann, 2008). Energy is absorbed or emitted by matter in discrete quanta called photons. The absorption of light leads to an electronic excitation (ground state to excited state), promoting an $n \rightarrow \pi^*$ or $\pi \rightarrow \pi^*$. However, a large variety of photoreactions with high selectivity, chemical yields and photon efficiencies have been developed. Due to its easy generation, control and handling, light is also considered as a clean and traceless reagent. Despite the above mentioned advantages, photochemical reactions for chemical production or R&D processes are quite rare and most technical processes are limited to commodity chemicals (Oelgemöller and Shvydkiv, 2011). The UV-light having a wavelength ranging between 260-400 nm (equivalent to the UV-A and UV-B ranges) can modify particle size of 3 nm by causing their surface plasmon resonance (SPR) to decrease at a rate which increases with the photon energy (Gorham et al., 2012).

AgNP is the preferred nanomaterial for SERS surfaces because of the following reasons. Copper easily oxides and hence not stable. Typically, nanostructured plasmonic materials, like gold (Au), silver (Ag) and copper (Cu), are used for the preparation of SERS substrates. However, application of Cu is limited since the material undergoes rapid oxidation in air. Gold and silver are, on the other hand, most widely used due to their high stability compared to copper and most importantly they have LSPR frequencies in the visible to near infrared range where most of the Raman scattering occurs. Another important consideration is the energy at which loss due to interband transitions starts to occur. eV is the total damping rate due to electron-ion scattering, electron-electron scattering, and inelastic scattering from defects and grain boundaries. In Au and Cu, interband transitions become important at 2.3 and 2.1 eV, respectively, meaning that Au and Cu can support low-loss Surface Plasmonic Polariton (SPP)'s propagation only in the infrared regime. Interband transitions prevent all but Ag from being useful for SPP devices in the visible range. The interband transition energy for Al is even lower, at 1.41 eV. Among the alkali metals, Na and K exhibit low losses comparable with those of Ag. However, the challenges of probing these materials in vacuum and the impossibility of out-of-vacuum device applications have limited the research that has been done in this area.

The synthesis of AgNPs can be done using a bottom-up approach. This approach provides an opportunity to produce AgNPs in a range between 1-100 nm. It also has the advantage of producing stable AgNPs compared to AgNPs produced by the top-down approach, because the nanoparticles are formed as defined crystalline structures. The stability of nanoparticles is of significance when examining and exploiting their properties. The AgNPs can be synthesised through chemical reduction, photochemical reduction, metallic wire explosion, sonochemical methods and polyol methods. The simplest and the most commonly used method for metal nanoparticles is the chemical reduction of metal salts.

A reasonable way to prepare AgNPs with tunable size is to choose a reductant with suitable reactivity to control the nucleation growth processes of the particles (LaMer and Dinegar, 1950). The chemical reduction method involves the reduction of the metal salts in the presence of an appropriate capping

agent, which is essential for controlling the growth of metal colloids and preventing them from agglomerating. Synthetic polymers such as polyvinylpirrolidone (PVP), polyvinylalcohol (PVA) and gelatin are often used as protecting agents. The reduction of silver nitrate with alkyl acid phosphate in the presence of gelatin can produce particle sizes of 0.1-1.0 nm. It has been shown that vinyl polymers possess better protecting characteristics than substances such as gelatin (Nersisyan et al., 2003). The reduction of pre-heated silver nitrate solution in the presence of PVP results in silver powder with a particle size of ~300 nm. The size of these particles can be reduced to 100 nm, if the reduction of silver nitrate is done by hydrazine in the presence of PVP. An aqueous solution of 0.01M silver nitrate in the presence of PVP and PVA reduced with formaldehyde permitted the formation of colloidal dispersion of silver with a particle size ranging from 7 to 20 nm (Kan-Sen and Chiang-Yuh, 2000). It was noted that a low concentration of silver solution with a large quantity of PVP (Ag/PVP ¼ 1/1-9), negativity influenced the quality of the product and efficiency of the process (Nersisyan et al., 2003). Other reducing agents such as citrate (Pillai and Kamat, 2004) and ascorbic acid (Sondi et al., 2003) are among the most widely used reductants for the reduction of silver nitrate in aqueous solutions. AgNPs prepared with citrate produced spherical and rod-like silver particles due to the competition of nucleation and growth processes (Dong et al., 2009) and those prepared by borohydride produced small spherical AgNPs (less than 10 nm) due to the high reactivity of the reducing agent which induced an explosive nucleation process. The change of reaction parameters such as molar ratio of the reductant or silver precursor, pH, or temperature of the reactions when using citrate or borohydride, affects the nucleation and growth processes and thus the size of the silver nitrate is not well controlled (Qin et al., 2010). When synthesising gold nanoparticles by reducing $HAuCl_4$ with citrate, variation in molar ratio of citrate/$HAuCl_4$ or pH is effective to tune the reactivity of the gold precursor (Ji et al., 2007). In addition, it is easy to mediate the growth stage and nucleation and prepare gold nanoparticles with various sizes by changing the molar ratio or pH of the reactions.

The improvement of the adhesion properties of metal nanoparticles on solid supports should maintain the integrity and performance of SERS support stability over time (Park et al., 2013). Immobilisation of metal nanoparticles via electrostatic interactions has been found to be unstable in aqueous environments due to weak binding forces. The use of silver doped membranes in aqueous environment demands stable immobilisation of nanoparticles in order to reduce the risk of releasing a high load of nanoparticles into the aquatic environment (Yin *et al*., 2013). In the filtration process, the unstable immobilised metal nanoparticles on modified track etched membranes could easily leach through the membrane resulting in deterioration and loss of application (Park et al., 2013). Taurozzi and Tarabara (2007) reported the successful functionalisation of aminopropyltrimethoxysilane (APTMS) via amide bond formation on the surface of polycarbonate membranes and achieved the immobilisation of colloidal silver nanoparticles thereon via an amine-silver bond. Immobilised silver nanoparticles could resist applied hydraulic conditions although the nanoparticles were scratched off under mechanical stress (Taurozzi and Tarabara, 2007). Andrade *et al*. (2010) immobilised silver nanoparticles on the surface of modified glass via APTMS, which was coupled via the siloxy linkage and the composite was used to detect Congo Red dyes by SERS. Siloxy linkage functionalised on modified glass surfaces used by

Andrade et al. (2010) was found to be unstable when exposed to various solvent conditions. The siloxy linkages undergo hydrolysis upon exposure to alkaline and high temperature environments (Goddard and Hotchkiss, 2007).

In summary, the review of literature has shown that the development of a stable SERS platform has not yet been fully achieved. Various challenges remain, namely to isolate solutes via their rapid separation from the environmental matrix in which they occur, the separation of different molecules in the concentrated mixture of diverse solutes, their preconcentration to detectable levels, their identification and quantification. Various methods have been developed for each of these steps but many methods are slow, cumbersome, require expensive equipment and skilled operators and cannot give a rapid evaluation. SERS is seen as a promising analytical technique but requires development of the platform upon which plasmonic resonance via stable and well-anchored nanoparticles can be maximized. Track etched polymeric membranes can be used to achieve nanofiltration of analyte droplets in order to trap the selected solutes on the surface of the SERS platform but will need a suitable separation technique such as capillary electrophoresis prior to the target molecule's immobilisation on or near the silver nanoparticles decorated upon the platform.

2 PREPARATION OF TRACK-ETCHED POLYETHYLENE TEREPHTHALATE MEMBRANE MODIFIED WITH AG NANOPARTICLES

2.1 INTRODUCTION

SERS platforms, usually coated with metal nanoparticle assemblies or nanostructured metal surfaces, are required for measurement of organic contaminants and the analyte molecules' activity defines the extent of SERS applications (Culha et al., 2012). Noble metal nanoparticles (gold, silver and copper) have been found to provide good SERS active substrates compared to other transition metals (Sharma et al., 2013). These noble metal nanoparticles enhance the Raman signal when molecules of analytes are attached or in close proximity to them (Schmidt et al., 2004). Effective adsorption of analytes on the support is critical when working with low concentrations of analytes such as pharmaceuticals (Smith, 2008). Among the noble metals, silver has been found to be the most commonly used metal nanoparticle. Silver nanoparticles have the highest surface plasmon resonance in an easily accessible spectra region, i.e. visible or near infra-red region (Muehlethaler et al., 2015). Immobilisation of SERS-active nanoparticles on stable supports such as membranes, improves reproducibility of the surface enhanced Raman spectroscopy (Taurozzi and Tarabara, 2007).

This chapter provides a description of chemicals and materials used to achieve the goals of the research. The chapter also gives information on methodology and characterization techniques used in the modification of polymer membrane and synthesis of nanoparticles.

2.2 EXPERIMENTAL AND ANALYTICAL TECHNIQUES

The method applied in this chapter was direct immobilisation of silver nanoparticles on amine treated track etched polyethene terephthalate membrane. The modification of the track-etched terephthalate membrane was via wet chemistry methods which are commonly applied in biotechnology studies where proteins or enzymes are immobilised on the modified membranes. All amine modifications of the membrane were conducted at ambient conditions unless otherwise stated.

2.2.1 CHEMICALS AND MATERIALS

The following chemicals and materials were used in this study: track-etched polyethylene terephthalate (PET) membrane (thickness of 23 µm, pore density of 4.5×10^8 tracks/cm^2, pore diameter of 0.2 µm, asymmetric pores) obtained from Flerov Laboratories, JINR, Dubna Russia; stable colloidal silver solution obtained from Flerov Laboratories, JINR, Dubna Russia; ethylenediamine (EDA) 99%; diethylenetriamine (DETA) 98%; N-ethyl-N(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC);

N-hydroxy succinimide (NHS); absolute ethanol (EtOH) 99.9%; silver nitrate ($AgNO_3$) 98%; trisodium citrate ($Na_3C_6H_5O_7$) 98%; sodium sulphate (Na_2SO_4) 99%; sodium carbonate (Na_2CO_3) 99%, propanethiol 99%, poly ether imide (PEI), hydrochloric acid (HCl) 37%. All chemicals were purchased from Sigma-Aldrich except DETA and EDA, which were purchased from LGCC group. All aqueous solutions were prepared by using de-ionised water.

2.2.2 METHODS

2.2.2.1 Preliminary Coating of Colloidal Silver onto the Track-Etched Polyethylene Terephthalate (PET) Membrane

A preliminary coating of silver onto a PET (thickness of 23 µm, pore density of $4.5x10^8$ tracks/cm^2, pore diameter of 0.2 µm, and asymmetric pores) was carried out using colloidal silver solution obtained from Flerov Laboratory, JINR, Dubna (Russia).

PET membrane (dimension 4x2 cm^2) was soaked in 0.05% of poly ether imide (PEI) solution for 1 h at room temperature. Thereafter, it was rinsed with ultra-pure water. The modified PET-PEI was then soaked in 0.001 M of HCl solution for 1h at room temperature. Then it was rinsed with ultra-pure water. The obtained membrane was soaked in the colloidal silver solution for 12, 24, 36 or 48 h. The prepared samples (PET, PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag(36 h) and PET-PEI-Ag(48 h)) were characterized using UV-Vis and SEM. Their contact angle was also determined.

2.2.2.2 Modification of surface of polymer membrane

The surface modification of polyethene terephthalate track-etched membranes involved the following experimental steps: functionalisation of the surface with the amino group via an amide bond; immobilisation of silver nanoparticles on the surface via silver-nitrogen covalent bond. The schematic reaction protocol in Figure 2.1 shows the surface modification and immobilisation of silver nanoparticles on track-etched PET membrane.

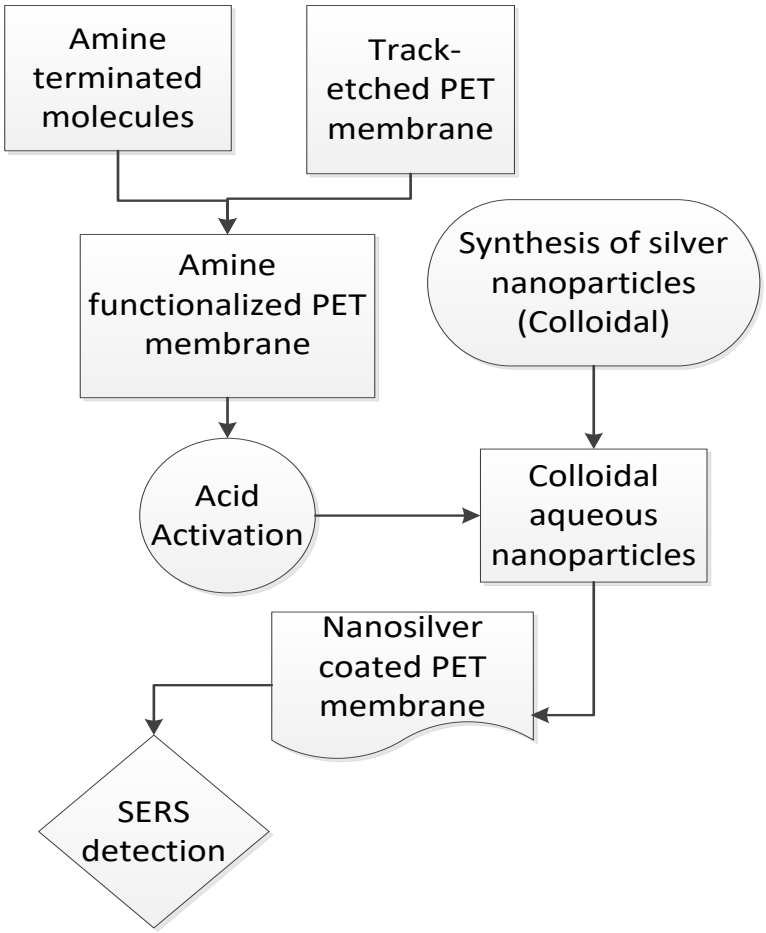


Figure 2.1: Schematic experimental protocol for surface modification of PET

2.2.2.3 Aminolysis of the PET membrane

The surface of PET membrane was modified by immersing samples of 2 x 2 cm pieces of track-etched PET membrane in a 100 mL aqueous solution of diethylenetriamine (DETA). The aqueous DETA concentrations of 45%, 60%, and 75% (v/v) were used. The exposure of PET membranes to amine solution was carried out at ambient temperature (approximately 23°C) for 12, 18 and 24 hours. Sodium carbonate was used as a catalysing agent to stabilise the formation of an amide bond.

The aqueous solution of 1 mM sodium carbonate (2 mL) was added to an aqueous solution of DETA. The reaction at the solid-liquid interface was left to run for a given time under agitation. After immersion of PET membrane in an aqueous solution of DETA, the membrane was doubly washed with distilled water and absolute ethanol to remove physically adhered DETA from the surface of PET membrane. The rinsed PET membrane was air-dried at room temperature for 24 hours. The modified and unmodified PET membranes were characterised by Fourier Transform Infrared (FT-IR) and X-ray photoelectron spectroscopy (XPS) to confirm the formation of the amide bond and confirm the presence of the amine group on the surface.

2.2.2.4 Silver nanoparticles immobilisation

The synthesis and immobilisation of silver nanoparticles on the surface of the modified surface of PET membrane was also done by immersing the DETA treated track etched PET membranes in the preheated silver nitrate solution to which trisodium citrate had been added. The immobilisation of silver nanoparticles on the surface was simultaneously with the synthesis of silver nanoparticles that occurred via reduction of silver nitrate by trisodium citrate.

Specifically, a solution of silver nitrate (100 mL, 1mM) was heated to a temperature of 90°C. Thereafter an aqueous solution of trisodium citrate (1 mg/100 mL) was added to the preheated silver nitrate solution followed by immersion of the track etched PET membrane. The volumes of trisodium citrate added to silver nitrate solution were 1 mL, 2 mL or 3 mL. The reduction of silver nitrate was carried out for 10 minutes, 15 minutes, 20 minutes or 30 minutes. The silver nanoparticle decorated PET membrane was rinsed twice with distilled water to remove physically adsorbed nanoparticles on the surface membranes. Then the silver decorated PET membrane was air-dried at room temperature for 24 hours.

The synthesis of silver nanoparticles was confirmed by UV-vis spectroscopy of the silver nanoparticles solution after reduction of silver nitrate. The shape and sizes of silver nanoparticles were characterised by transmission electron microscope. The surface morphology of silver decorated PET membrane was characterised by scanning electron microscopy and the silver nanoparticles on the modified PET membrane were characterised by x-ray photoelectron spectroscopy. The amount in percentage of silver nanoparticles was shown by energy dispersive spectroscopy (EDS).

2.2.2.5 Synthesis of silver nanoparticles

The silver nanoparticles were furthermore synthesised using a modified wet chemical method by Lee and Meisel (1982). Silver nitrate was used as a silver salt precursor and trisodium citrate served both as a reducing and stabilising agent. The silver nanoparticles were synthesised by bringing to 90°C, a 100 mL of 1 mM of aqueous silver nitrate. When the aqueous solution of silver nitrate reached 90°C, aliquots of 1 mL, 2 mL, or 3 mL aqueous solution of trisodium citrate (1 mg/100 mL) were slowly added dropwise. Then the mixture was left to react for 10, 15, 20 or 30 minutes. Thereafter, the solution was left to cool to ambient room temperature (23^{o}C). Details of the experimental conditions and codes are presented in Table 2.1.

Table 2.1: Experimental parameters and sample codes for modification of polyethylene terephthalate membrane and synthesis of silver nanoparticles.

Aminolysis of polyethylene terephthalate (PET) membrane			
Parameters	Time/Hours	Concentration%	Sample code
Time of reaction	12 hours	DETA 60%	12-APET
	18 hours	DETA 60%	18-APET
	24 hours	DETA 60%	24-APET
Concentration of DETA	18 hours	DETA 75%	75A-PET
	18 hours	DETA 60%	60A-PET
	18 hours	DETA 45%	45A-PET
	0 hours	DETA 0%	Co-APET

Synthesis of silver nanoparticles (optimisation)				
Parameters	Volume	Time	Temperature	Sample Code
Volume of trisodium citrate	1 mL	20 min	75°C	1-TriNa
	2 mL	20 min	75°C	2-TriNa
	3 mL	20 min	75°C	3-TriNa
Time of reduction reaction	2 mL	10 min	75°C	10-AgNP
	2 mL	20 min	75°C	20-AgNP
	2 mL	30 min	75°C	30-AgNP
Temperature of reduction reaction	2 mL	20 min	90°C	90C-Ag
	2 mL	20 min	75°C	75C-Ag
	2 mL	20 min	60°C	60C-Ag

Table 2.2: Experimental parameters and sample codes for preparation of SERS membranes

Immobilisation of silver nanoparticles on modified track-etched PET membrane				
Parameters	Volume	Time	Temperature	Sample code
Time of immobilisation	2 mL	10 min	90°C	10-AgPET
	2 mL	15 min	90°C	15-AgPET
	2 mL	20 min	90°C	20-AgPET
	2 mL	25 min	90°C	25-AgPET
	2 mL	30 min	90°C	30-AgPET
			Control	Co-AgPET

2.2.2.6 Characterisation Techniques

The main characterisation techniques used to achieve the goals of the research included: Fourier transformation infrared (FT-IR) for determination of changes in functional groups on the membrane surface, UV-Vis for determination of plasmonic peaks for confirmation of formation of silver nanoparticles (AgNPs), transmission electron microscopy (TEM) for shape and size of synthesised AgNPs, scanning electron microscopy (SEM) for PET membrane surface morphology analysis.

The modified and unmodified PET membranes were characterised by FT-IR to confirm ester bond scission on the surface of the modified membrane and formation of a new amide bond. The FT-IR spectra of the modified and unmodified membrane samples were attained using Perkin Elmer model

Spectrum Two spectrometer within a range of 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} and at 20 scans.

The surface morphology of the PET membrane was characterised using Zeiss Auriga SEM. The samples were sputter coated with carbon using Quorum T15OT for 5 minutes prior to characterization. The sputter coating was to make the polymer membrane conductive.

3 RESULTS AND DISCUSSION

3.1 Preliminary coating of colloidal silver onto the track-etched polyethylene terephthalate (pet) membrane

In this subsection the samples soaked in 0.05% of poly ether imide (PEI) as made in Section 2.2.2.1 and then exposed to colloidal silver are presented. Figures 3.1 and 3.2 present the photographic images and UV-Vis results of PET, PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag(36 h) and PET-PEI-Ag(48 h).

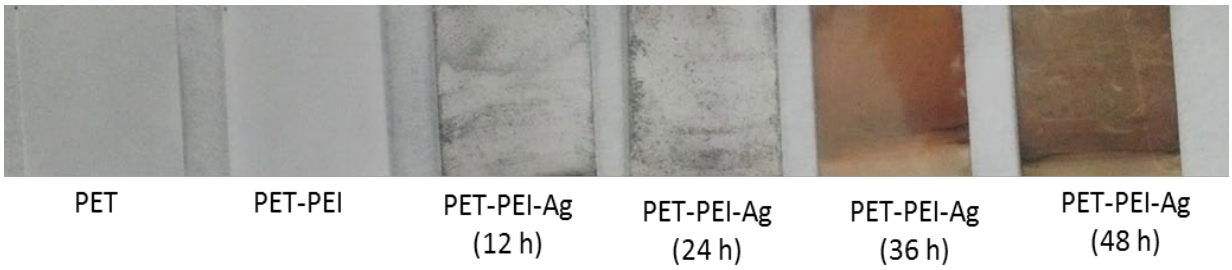


Figure 3.1: Image of PET, PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag(36 h) and PET-PEI-Ag(48 h).

Figure 3.1 shows that silver spots were visible on the modified PET-PEI after soaking it in the colloidal silver solution for 12 and 24 h. A silver layer was observed on the modified PET-PEI after soaking it in the colloidal silver solution for 36 and 48 h. Figure 3.2 presents the UV-Vis spectra of PET, PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag(36 h) and PET-PEI-Ag(48 h).

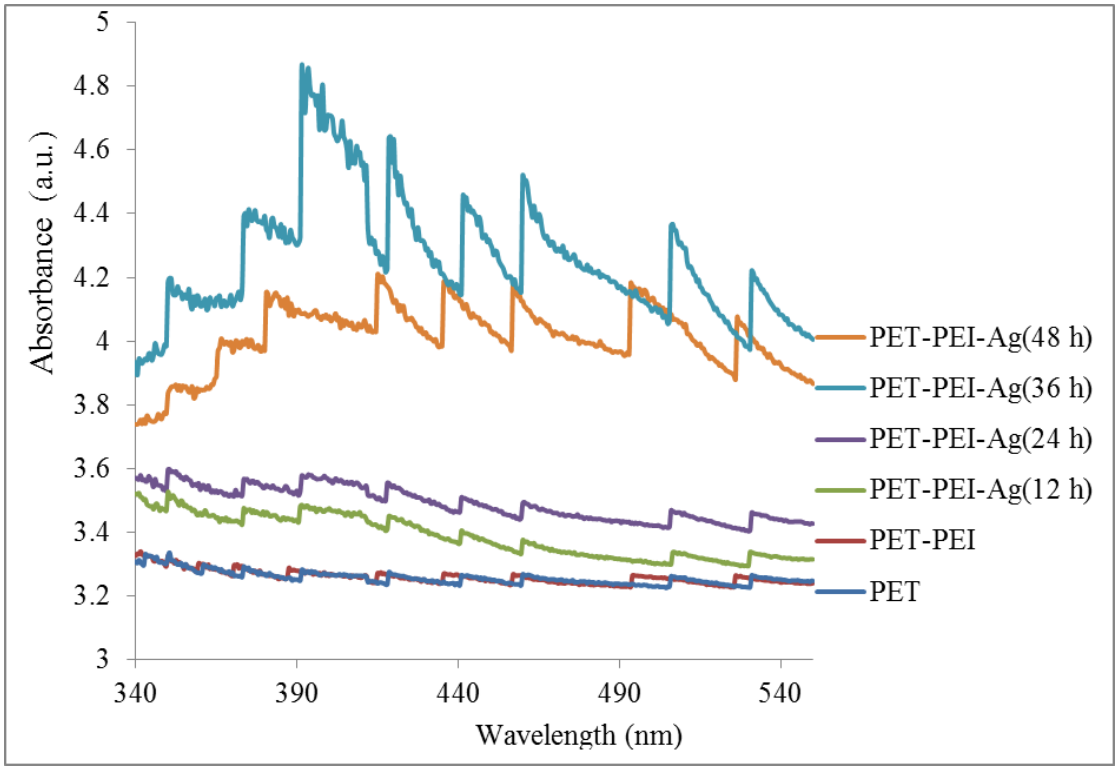


Figure 3.2: UV-Vis spectra of PET, PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag (36 h) and PET-PEI-Ag(48 h).

The UV-Vis spectra in Figure 3.2 show that a characteristic absorption peak of silver nanoparticles appeared around 440 nm. The silver peak observed in PET-PEI-Ag (36 h) and PET-PEI-Ag (48 h) spectra corroborated the silver layer that was observed when PET-PEI was soaked in colloidal silver solution for 36 and 48 h (Figure 3.2). The spectrum of PET itself had no UV-Vis signals in the measured region because it is neither transparent nor liquid. The same observation was made by Yang et al. (2013). They reported that the PET membrane does not absorb between 300 and 800 nm, which is in agreement with the PET spectrum that is reported in this study. Sithole (2015) reported that the UV-Vis peak characteristic of silver nanoparticles appears around 450 nm as shown in Figure 3.2.

Figure 3.3 presents the SEM images of PET, PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag(36 h) and PET-PEI-Ag(48 h).

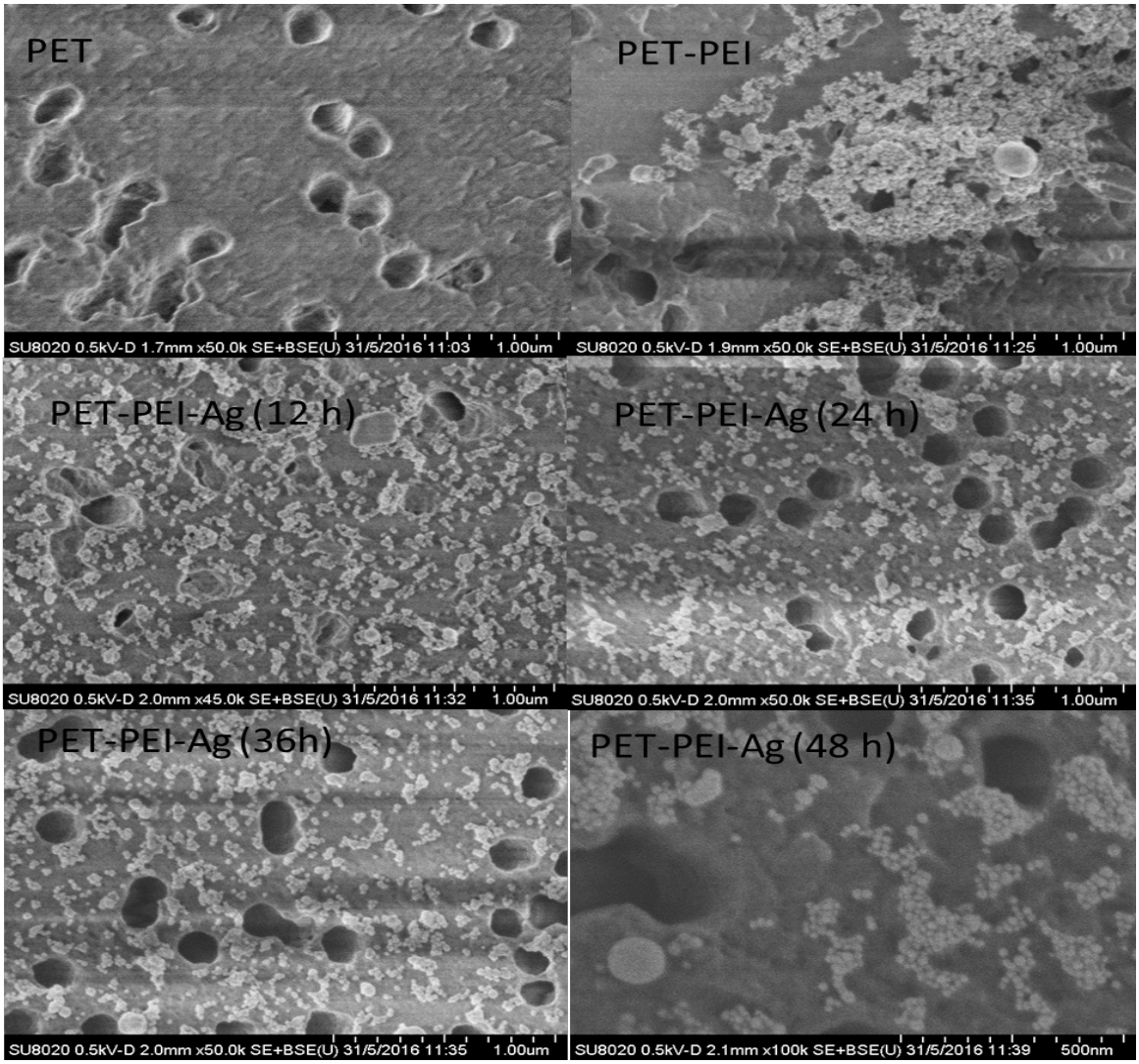


Figure 3.3: SEM image of PET, PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag(36 h) and PET-PEI-Ag(48 h).

The pore diameter of PET (Figure 3.3) was determined using ImageJ software and was found to be 0.21±0.03 µm, which corroborated the pore diameter of 0.2 µm of PET given by the supplier. Figure 3.3 shows that the PET itself was smooth with well-defined track etched pores, and PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag(36 h) and PET-PEI-Ag(48 h) were all covered by small Ag particles in the nanoscale, without clogging the pores, but which did not uniformly coat the surface of the membrane. Table 3.1 presents the contact angle measurements of PET, PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag(36 h) and PET-PEI-Ag(48 h).

Table 3.1: Contact angle of PET, PET-PEI, PET-PEI-Ag(12 h), PET-PEI-Ag(24 h), PET-PEI-Ag(36 h) and PET-PEI-Ag(48 h).

Sample	Contact angle
PET	46.9±5.9°
PET-PEI	58.6±9.7°
PET-PEI-Ag(12 h)	61.9±6.1°
PET-PEI-Ag(24 h)	74.1±5.0°
PET-PEI-Ag(36 h)	81.2±4.2°
PET-PEI-Ag(48 h)	81.6±1.6°

It was observed that the contact angle of the as-received track-etched PET membrane was 46.9±5.9° (Table 3.1). The contact angle increased as the track-etched PET was treated with PEI and colloidal silver solution (for 12, 24, 36 or 48 h), which reduced the wettability of the track-etched PET and may have an impact upon analyte retention. Korolkov et al. (2014) reported that the track-etched PET (thickness of 23 μm, pore density of $4.3x10^7$ tracks/cm^2, pore diameter of 0.55 μm) had a contact angle of 43.0±1.4°, which was slightly different from the contact angle of 46.9±5.9°, reported in the current report and can be due the difference of the PET properties and etching conditions. Overall, the strategy of immobilisation of ready-made colloidal silver upon PET-PEI did not lead to very good surface coverage of the PET trackmembrane with nanoparticles, thus the next section details the results of the DETA modified materials.

3.2 Modification of membrane

3.2.1.1 Aminolysis of PET membrane

The following experimental results are for the wet chemistry modification of the PET membrane with diethylenetriamine (DETA) as set out in Section 2.2.2.2 and Table 2.1. The pieces of PET membrane were exposed to the amine solution by immersing them in varying DETA concentrations (percent volume per volume) and at different exposure times at room temperature (23°C). Equal size pieces of PET membrane were cut in order to expose the surface area of the membranes equally and monitor the effects arising from different concentrations and reaction times. All these conditions were chosen in order not to cause serious degradation to the PET membranes.

3.2.1.2 Immobilisation of Silver nanoparticles on PET membrane

Silver nanoparticles were prepared from silver nitrate as a silver salt precursor and trisodium citrate as a reducing and stabilising agent. The PET membrane modified with amine and activated with hydrochloric acid was immersed simultaneously with trisodium citrate in a pre-heated solution of silver

nitrate. In the course of reduction of silver nitrate to silver ions and then silver nanoparticles, the nanoparticles were immobilised on the surface of PET membrane.

The mechanism of the reduction of the silver nitrate by citrate can be expressed as follows:

$$4\,Ag^{+} + C_6H_5O_7Na_3 + H_2O \longrightarrow 4\,Ag^{0} + C_6H_5O_7H_3 + 3\,Na^{3+} + H^{+} + O_2 \uparrow$$

Observed colour changes were noted where PET membrane pieces changed from the whitish colour of track-etched PET membrane to yellow orange then brown, which showed that silver nanoparticles were being deposited on the surface. It was noted that as the number of silver nanoparticles immobilised on the PET membrane increased, the colour of the acid-activated triamine modified PET membrane changed. The changes in the colour of the surface of PET membranes show an increase in immobilised silver nanoparticles with the time of reaction. This also relates to changes in the colour of the reaction solution from clear to pale yellow then orange and finally brownish grey.

The surface modification of PET membrane and immobilisation of silver nanoparticles directly on the modified PET membrane is presented in the schematic mechanism in Figure 3.4.

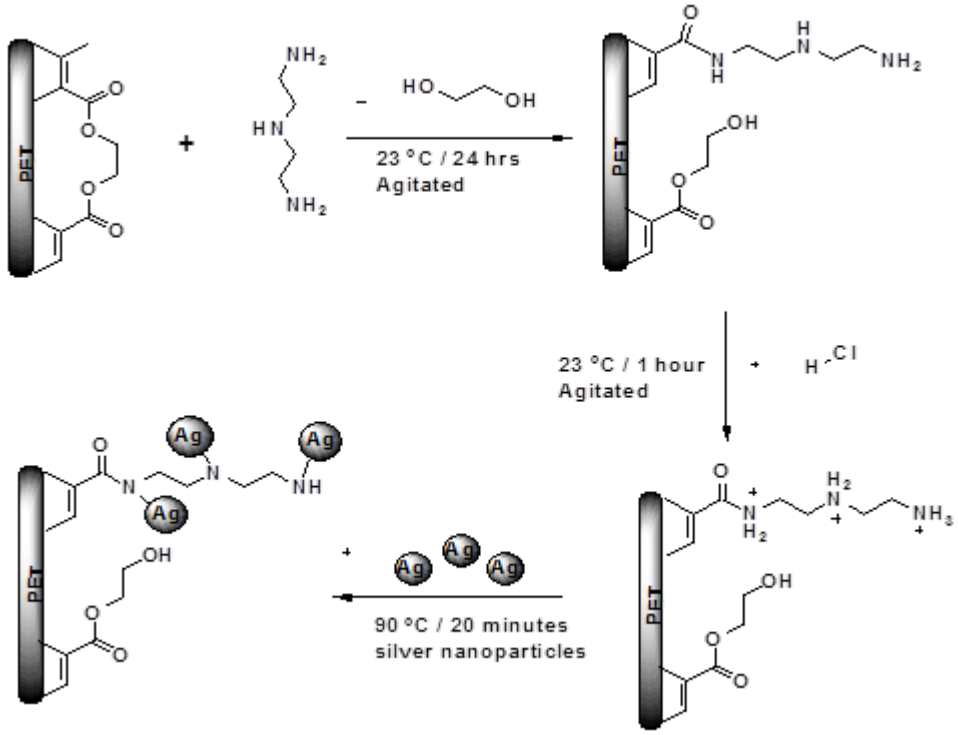


Figure 3.4: The mechanism of modification of PET membrane with DETA and immobilisation of AgNPs via DETA's nitrogen atom.

3.2.1.3 Fourier Transformation Infrared

The FT-IR was used to compare various wet chemistry treatments of PET membrane using triamine at different concentration in distilled water and at different times of immersion of the PET membrane in the aqueous triamine solution. As shown in Figure 3.4, an amide bond is introduced on the surface of the PET membrane via bond scission of the ester bond in polyethene terephthalate and formation of amide

(N-C) bond. The modification of PET membrane surface with amines results in the formation of amide bonds and the introduction of amines on the surface. These functional groups are therefore determined by characterising with Fourier transformation infrared.

The modification of the PET membrane by amines is dependent on variables such as the temperature of the reaction mixture, the concentration of the triamine and time of exposure of the PET membrane to triamine solution. In this study, only the concentration of triamine and duration of reaction were changed to determine the surface modification of the PET membrane. Studies show that treating the membrane with amines at high temperatures degrades its tensile strength (Nissen et al., 2008). The spectra of the unmodified and modified membrane based on time of modification at constant temperature and constant concentration of triamine are shown in Figure 3.5. The parts of the FT-IR spectra of interest are the amide bond vibrations which occur between 1500 and 1700 cm^{-1} wavenumbers and amine stretching occurring between 3400 and 3500 cm^{-1}. This is due to the introduction of the amines on the PET membrane surface via formation of amide bonds by the carbonyl carbon and nitrogen of amine. The additional presence of the ethylene moieties on the surface of modified PET membrane could be supported by changes in transmittance of spectra at 2900 cm^{-1}.

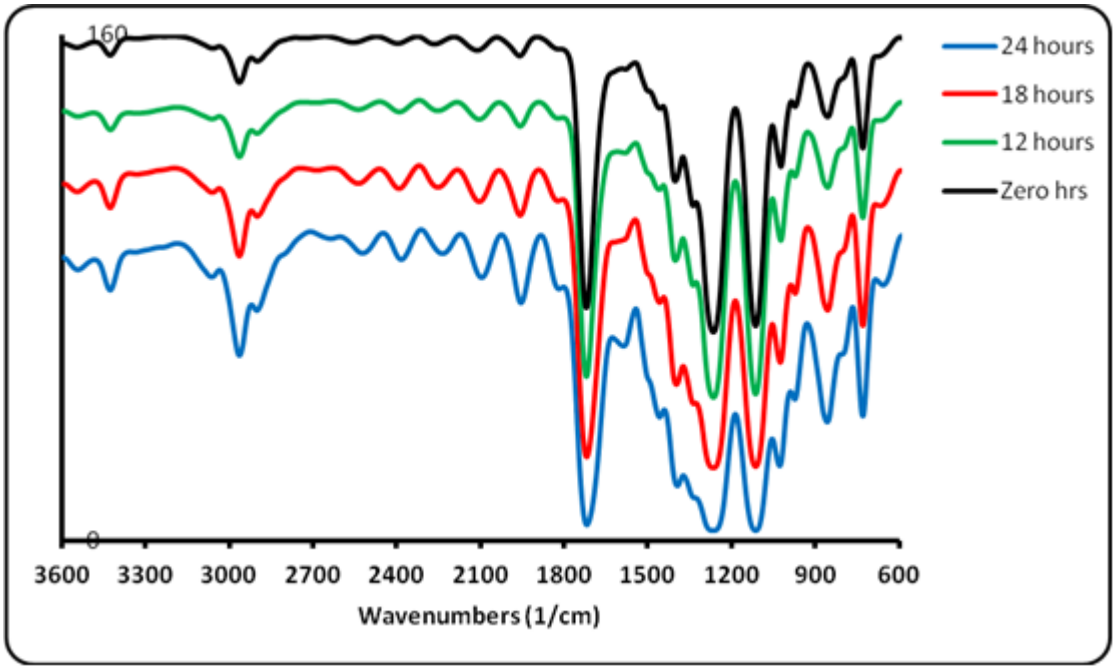


Figure 3.5: Spectra of unmodified PET membrane (black), 6 hours of PET exposure to triamine (green), 18 hours of exposure (red) and 24 hours exposure (blue).

The FT-IR results show that there was a change of transmittance between 1500 and 1700 cm^{-1} mainly for C-N bond stretching in the case of the PET membrane that had been exposed to triamine for 18 and 24 hours. Another notable change was noted between 3400 and 3500 cm^{-1} which are assigned to newly introduced amine N-H stretching and also the peak centred at 2900 cm^{-1} from extra ethylene C-H bond stretching. The change in transmittance is not very evident but this is consistent with observations made by Irena et al. (2009), where the change was attributed to aliphatic amines in the range 3400 to

3500 cm^{-1}. The notable change in the spectra is where the amide bond I and II are found between 1500 and 1700 cm^{-1}. The FTIR spectra of the PET membranes that were exposed for 24 hours to amine solution clearly show the amide I and II bonds as reported by Drobota et al. (2013). The spectra changed with time of exposure; the longer the time of exposure of PET membrane to amine solution, the more noticeable the peaks assigned to the above functional groups.

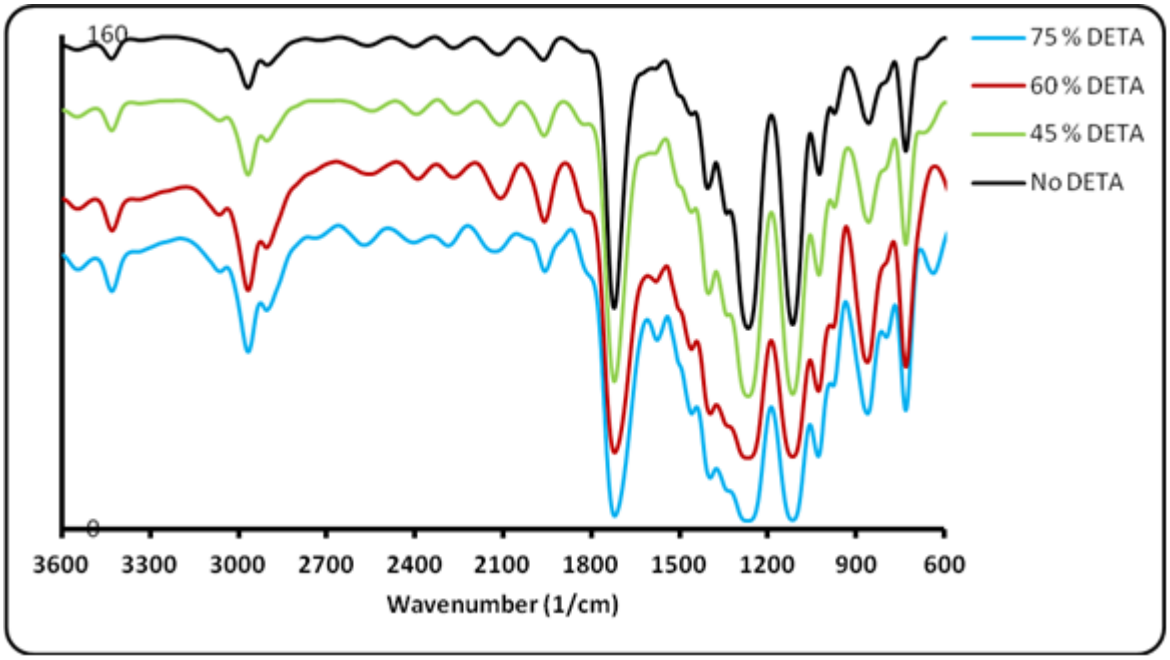


Figure 3.6: FT-IR spectra of unmodified and DETA modified PET membranes at a different concentration of amine: unmodified PET (black), 45% aqueous DETA (green), 60% aqueous DETA (red) and 75% aqueous DETA (blue).

The pronounced change in transmittance relates to the higher concentration of DETA as shown in Figure 3.6. Similar transmittance percent changes are observed in the region 3400 to 3500 cm^{-1} and functional groups signals were observed as was expected from the introduction of the amines and formation of amide bonds. The N-H stretching (both symmetric and asymmetric) peaks were not specifically observed between 3400 and 3500 cm^{-1}, but the change in transmittance percentage is contributed to by the presence of amine functional groups. The amide I and II bands could be observed in the case of the PET membrane that was subjected to aminolysis using DETA in distilled water but not as separate transmittances as reported by Xue and Lu (2013). The aqueous solution of DETA with 75% w/v resulted in Amide I and II bands for stretching vibrations v(C=O) overlapping each other appearing between 1500 and 1600 cm^{-1}. This could be caused by the lack of sensitivity of the equipment used, where the amide I and II bands appeared together. The presence of amide I and II bands on FT-IR spectra of the PET membrane subjected to DETA at different concentrations confirms that the surface of PET membrane was modified. The DETA concentrations higher than 75% resulted in degraded PET membranes that easily tore when held with tweezers and during washing with distilled water, thus the concentration should be controlled. Aminolysis of the PET surface was reported to be

feasible using low molecular weight amines such as ethylenediamine (EDA) and high molecular weight amines such as tetraethylenepentamine (Irena et al., 2009, Drobota et al., 2015, Nissen et al., 2008).

3.2.1.4 X-ray Photoelectron Spectroscopy

The X-ray photoelectron spectroscopy (XPS) results complement those of the FTIR, where an observed introduction of amine and amide functional groups was detected. The XPS results indicate that the unmodified and amine-modified PET membrane surfaces have the following elemental compositions as outlined in Table 3.2.

Table 3.2: Elemental percentages on the surface of unmodified, amine-modified and silver-coated PET membrane

PET Membrane	**Atomic percentages**		
	Carbon (C 1s)	Oxygen (O 1s)	Nitrogen (N 1s)
Unmodified	73.35	26.65	≈0
DETAModified	70.37	20.45	4.73

The XPS results in Table 3.2 show that carbon (C 1s) and oxygen (O 1s) were present on the surface of unmodified PET membrane. The amine-modified PET membrane shows the presence of nitrogen (N 1s) from the diethylenetriamine (DETA) that has replaced some oxygen that has been displaced via the aminolysis process, which involves ester bond scission and formation of an amide bond. The surface of amine-modified PET membrane has amine and amide moieties, whose nitrogen is prominently present as shown in Table 3.2. This shows that the scission of the ester bond and formation of amide bond was successful. The elemental compositions are also presented in the general survey taken for both PET membranes according to Figure 3.7. The general survey shows elements within the detection limits with significant amounts based on the chemical composition of the PET membrane. The XPS general survey graphs in Figure 3.7 are indicative of the change in elemental composition following modification of the surface with diethylenetriamine.

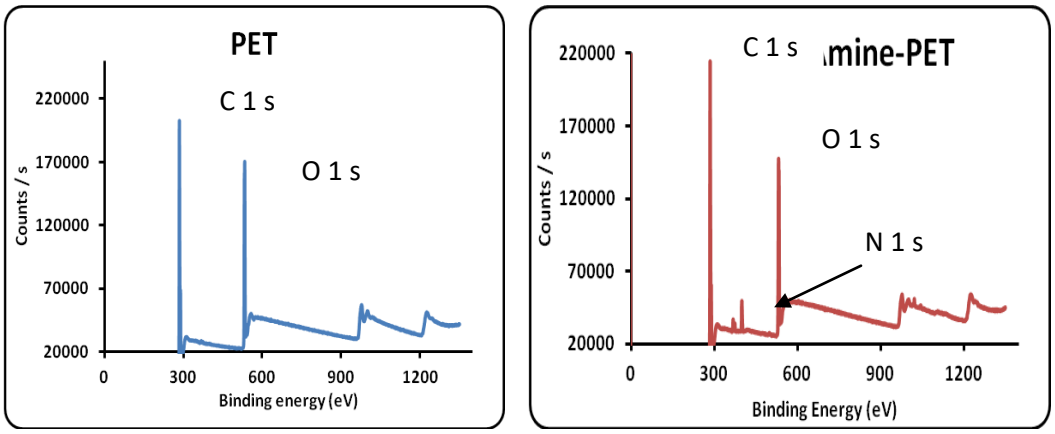


Figure 3.7: General survey of unmodified PET (PET) and amine-modified PET (Amine-PET) showing elements within detection limits

The general survey also shows that peaks of C 1s, O 1s and N 1s are in binding energy regions of 286, 532 and 400 eV respectively which agree with those binding energies reported in the literature (Xue and Lu, 2013). The survey shows that although nitrogen was present on the surface of the amine-modified PET membrane, it does not indicate the chemical state of the existing nitrogen on the surface.

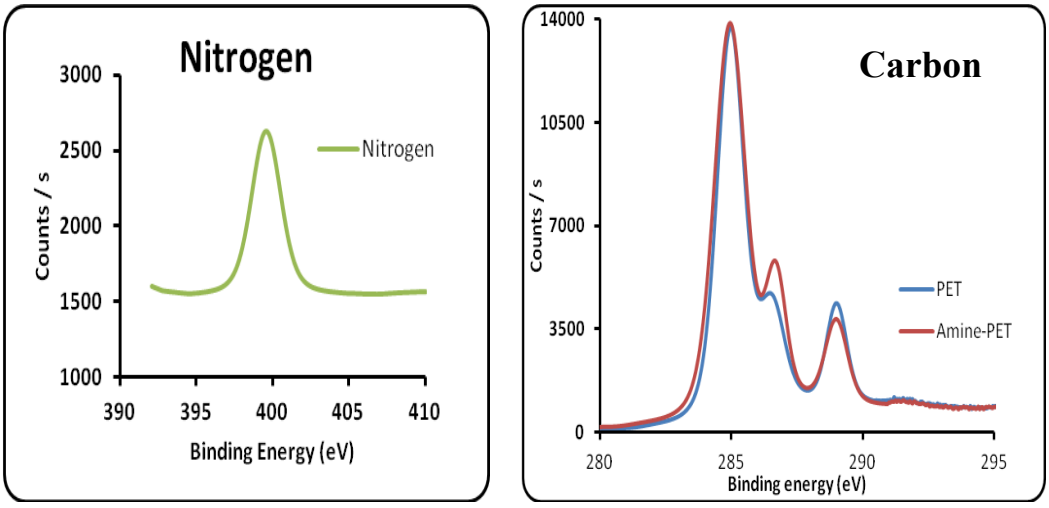


Figure 3.8: XPS spectra of N1s peaks of amine-modified PET and C1s peaks for both unmodified (blue) and amine-modified (red) PET membrane

Further to the general survey, the magnified N 1s shell in Figure 3.8, also show that the spectral peak for N 1s is at a binding energy of 400 eV which is for nitrogen in the chemical state of C-NH_2. Furthermore, the C 1s shell scan shown in Figure 3.8 shows three symmetrical peaks with binding energies at 284.9, 286.6 and 288.9 eV for the chemical states of C-C, C-O and O-C=O respectively (Vesel et al., 2008). These C 1s spectra peaks are indicative of a polymer membrane and satellite

peak π-π*, although not so pronounced at 291 to 292 eV, that is characteristic of a polyethene terephthalate polymer material (Xue and Lu, 2013). Figure 3.8 shows changes in peak heights of C-C and O-C=O, which are as a result of the introduction of more ethylene (C-C) moieties from the diethylenetriamine and formation of an amide bond (C-N) which replaces O-C=O to N-C=O. This is also presented in Table 3.3. The amide bond formation, therefore, results in a change in chemical state of carbon in O-C=O as observed in reduction of O-C=O peak height from 3428 to 2888 counts per second. The conspicuous change in C-O peak height is unusual as it is expected that a loss of glycol during the aminolysis reaction would result in reduction in height of C-O spectrum peak. This could be the contribution of carbon in this chemical state from the atmosphere during aminolysis reaction or storage.

Table 3.3: Peak height changes of chemical states of elements for unmodified and modified PET membrane.

Chemical State of elements	**Peak height (count/second)**	
	PET	Amine-PET
C-C	13158	13362
C-O	3209	4226
O-C=O	3428	2888

3.2.1.5 Thermogravimetric Analysis (TGA) of PET membrane

Thermogravimetric analysis was used to determine and compare changes in the thermal profile of the PET membrane samples. The analysis was used to quantify the weight loss relative to the applied temperature gradient. The comparison was amongst unmodified, amine-modified and silver-doped amine PET membranes, in order to monitor thermal degradation as a result of the modification process. The thermal profile is shown in Figure 3.9.

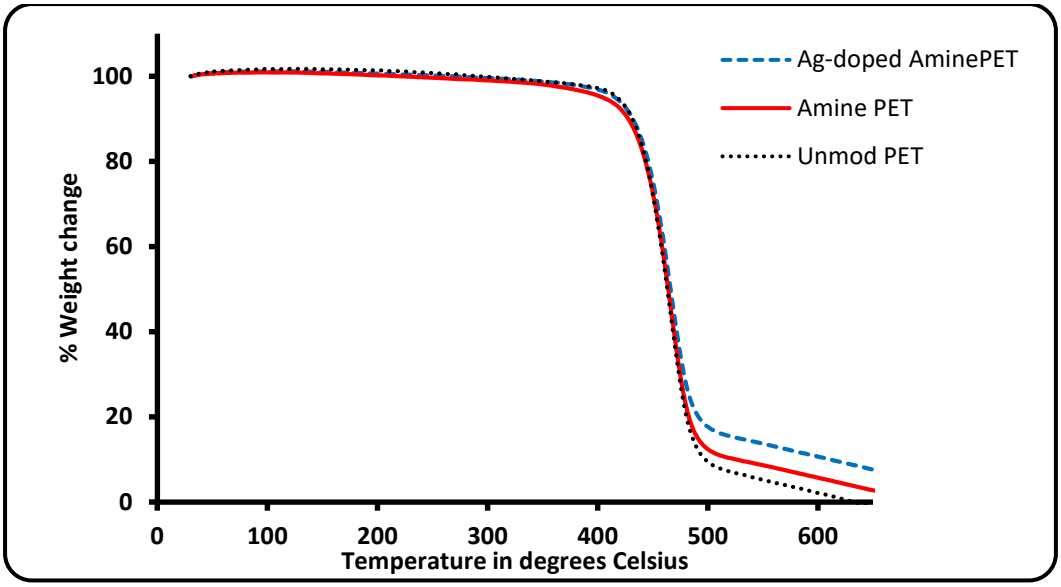


Figure 3.9: Thermal profile of unmodified PET, amine-modified PET and silver-doped amine PET

The thermal profile shows that all the membranes were stable, without weight loss up to 300°C where a 2.5% weight loss was observed until reaching a temperature of 390°C. After 390°C the rate of percent weight loss increased till reaching 490°C. In this range, an observed weight loss was from 97.5% to 10% for unmodified PET and 97.5% to 14% for amine-modified PET and weight loss was down to 20% for silver-decorated amine PET. The profile of weight loss gradient decreases sharply indicating a point where most of the volatile material has been lost and the remaining weight is the residue mainly carbon and silver for the silver-decorated amine PET. The different profiles after 490°C arose from different masses of mainly carbon and silver residues. The silver-doped aminePET shows 20% weight remaining, which is 10% more than unmodified PET and 6% more than amine-modified PET. The difference is due to the residual metalic silver which was still present after 490°C. The 4% difference between amine PET and unmodified PET is due to additional carbon from the diethylenetriamine functionalised on the PET membrane surface which correlated with the loss of ethylene glycol (two carbon atoms), as the introduction of DETA on the surface of PET results in addition of four carbons.

3.2.1.6 UV-vis of Silver nanoparticles

The structural characterisation of silver nanoparticles is widely performed by UV-visible spectroscopy. The silver nanoparticles having surface plasmon excitation exhibit intense absorption peaks within the 350 nm and 450 nm absorption band of the UV-visible region. The shifts in plasmon absorption peaks are due to the disparity in shape and size of silver nanoparticles depending on the volume/concentration of reducing agent used, time of reduction and temperature at which reduction occurred. The red shift indicates the increase in the size of silver nanoparticles and variations in size and shape of nanoparticles.

The results from UV-vis conducted on silver nanoparticles synthesised by reducing silver nitrate by trisodium citrate and simultaneously immobilising the nanoparticles on the modified amine-modified PET membrane surface at different temperatures are shown in Figure 3.10.

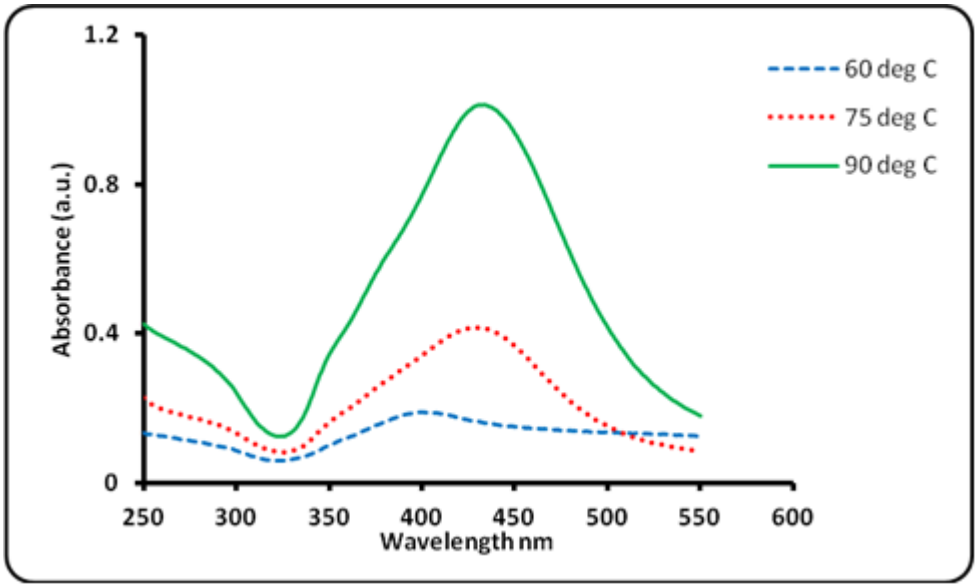


Figure 3.10: Plasmonic peaks of silver nanoparticles synthesised at different temperatures but at equal volumes of trisodium citrate at a constant time of 20 minutes. Plasmonic peaks: 1 mL trisodium citrate at 90°C (green); 1 mL trisodium citrate at 75°C (red); 1 mL trisodium citrate at 60°C (blue).

The plasmonic peaks decreased with a decrease in time at a fixed volume of trisodium citrate solution added to the salt precursor ($AgNO_3$) at constant temperature. It is also observed that upon reduction of the temperature at which silver nanoparticles were synthesised there is a reduced absorbance as shown by decreasing plasmonic peaks (Figure 3.10). The decrease in the temperature at which the silver precursor salt and the reducing/stabilising agent are heated correlates with the decrease in the peak of the plasmonic spectra. This shows that there is a more complete reduction of silver ions at higher temperatures rather than at lower temperatures. At a lower temperature at the same fixed time at a constant concentration, Figure 3.10 shows that complete reduction of silver ions is not achieved compared to the reduction at a higher temperature, in this case at 90°C. The plasmonic peak is at about 420 nm, which is typical of citrate-reduced silver nanoparticles due to the disparity in shape and size (Pillai and Kamat, 2004).

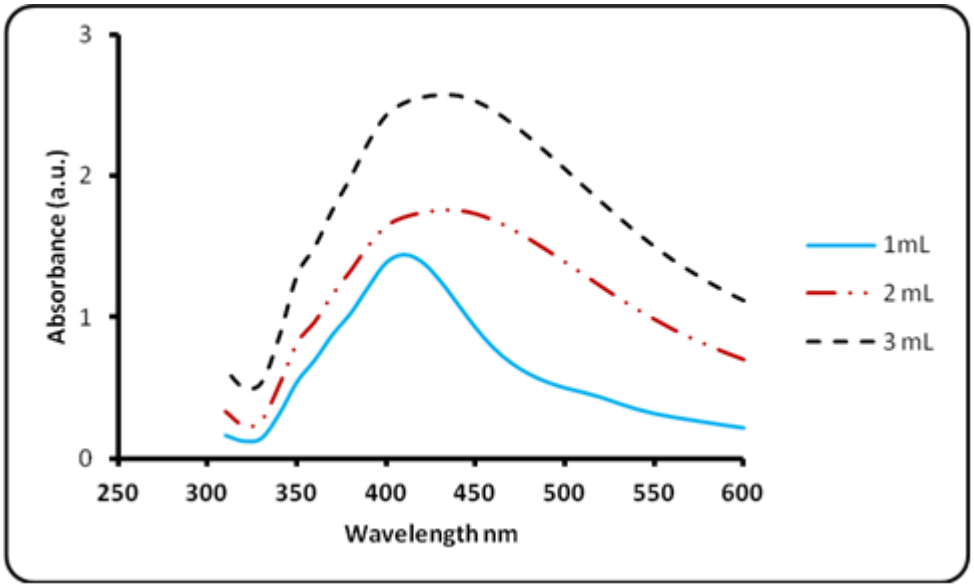


Figure 3.11: Plasmonic peaks of silver nanoparticles with varying volumes of trisodium citrate added to silver nitrate solution: 1 mL (blue), 2 mL (red) and 3 mL (black)

The increase in the volume of trisodium citrate added to the silver nitrate solution at pre-heated temperature of 90°C shows that there is a red shift in the plasmonic peak shown in Figure 3.11. At the same time, the plasmonic peaks become broader in shape, being characteristic of plasmonic peaks of silver nanoparticles synthesised by reduction with citrate. The blue-labelled plasmonic peak representing 1 mL of added trisodium citrate is narrow and occurs at 400 nm, this conforms to monodispersed silver nanoparticles peaks. This observation is supported by the literature reviewed (Bastus et al., 2014). This indicates that the lower the volume of trisodium citrate added, the narrower the peak and usually the smaller the nanoparticles, which are monodisperse. The addition of a high amount of trisodium citrate results in high nucleation of nanoparticles and therefore polydispersion, thus nanoparticles resulted that showed a disparity in both shape and size.

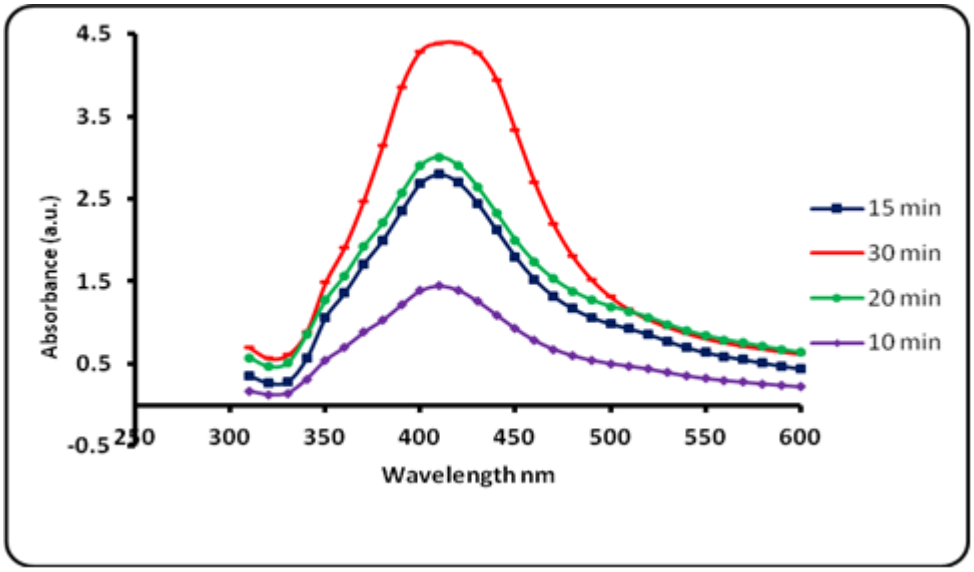


Figure 3.12: Plasmonic peaks of silver nanoparticles with varying reduction reaction time of silver nitrate and trisodium citrate solution at constant temperature and volume of trisodium citrate: 30 min (red), 20 min (green), 15 min (black) and 10 minutes (purple)

The plasmonic peaks in Figure 3.12 show that as time increased the concentration of silver nanoparticles increased and the peaks became narrower. The trend is in agreement with literature as regards the synthesis of silver nanoparticles, where they first form silver ions, then silver atoms, which later undergo nucleation to form silver nanoparticles dependent upon the time of reduction (Khan et al., 2011).

3.2.1.7 Transmission Electron Microscopy

The shape and size of the colloidal silver nanoparticles supplied by JINR, Dubna, Russia, were determined by transmission electron microscopy (TEM) analysis. The TEM characterisation was conducted in order to investigate the size, shape and dispersity of the colloidal silver nanoparticles. The TEM images indicate that the majority of the silver nanoparticles were spherical in shape with a few rod-like and triangular nanoparticles. This is in agreement with literature that silver nanoparticles synthesised via reduction of silver nitrate by trisodium citrate are polydispersed (Abou-El-Nour et al., 2010). The image in Figure 3.13 shows TEM images of the silver nanoparticles. The different shapes and sizes of silver nanoparticles that were synthesised agree with the UV-vis spectroscopy results whose plasmonic peaks are broad in shape depicting polydispersity of nanoparticles (Khan et al., 2011).

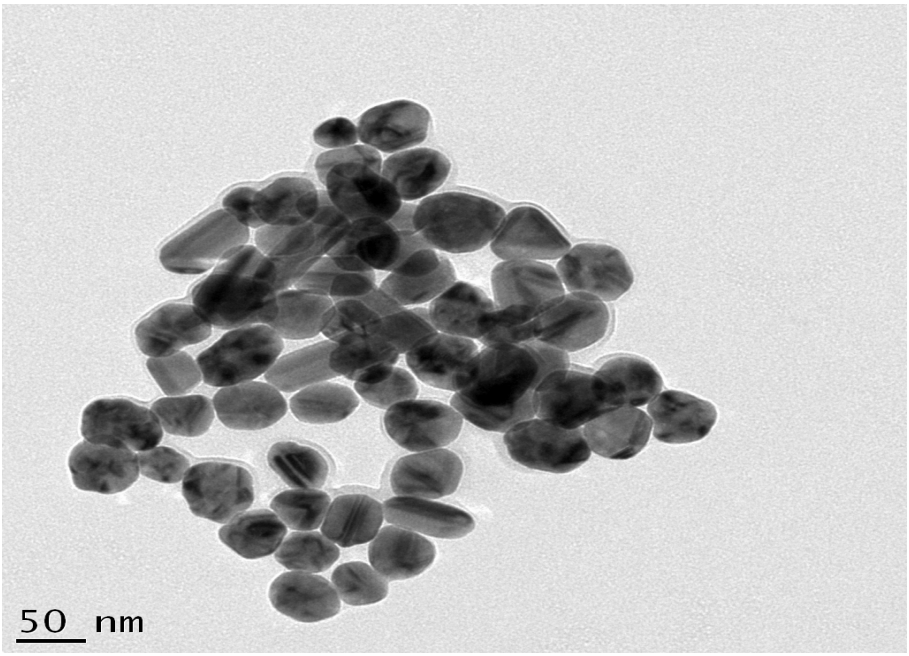


Figure 3.13: TEM images of colloidal silver nanoparticles

The TEM image in Figure 3.13 showed that the colloidal silver nanoparticles had an average silver nanoparticle size of 54 nm.

3.2.1.8 Scanning Electron Microscope

The SEM observations were furthermore carried out to observe the surface morphology regarding the distribution of silver nanoparticles on the surface of unmodified and modified PET membrane under different conditions. The varied conditions were: temperature for synthesising silver nanoparticles; the volume of added trisodium citrate, the duration of synthesis, acidic activation of PET membrane and time of exposure of PET membrane to triamine (DETA). The PET membranes (A, B and C), both unmodified and modified with amine were exposed to the reduction reaction of a precursor silver salt ($AgNO_3$) by a stabilising and reducing agent ($Na_3C_6H_5O_7$). The membranes were exposed to hydraulic forces by washing under water pressure to wash off any physically adsorbed nanoparticles. Figure 3.14 shows the SEM images of PET membranes subjected to different modification processes. In Figure 3.14A, the unmodified membrane that was immersed in the silver nitrate solution that was reduced by trisodium citrate is shown. The SEM micrographs Figure 3.14 B and C, show the membrane surface after modification with triamine and silver nanoparticles. The SEM micrograph Figure 3.14 C, shows that the membrane after it was activated in acidic media using 1 mM of hydrochloric acid. The acid activated membrane surface shows more homogeneous monolayer coverage of self-assembled silver nanoparticles as well as the track etched membrane pore distribution. The membrane surface of non-acid activated membrane shows sparse, homogeneous monolayer coverage of nanoparticles. In the case of the unmodified PET membrane, it was observed that no silver nanoparticles were chemisorbed on the surface although immersed in a reaction mixture where silver ions were reduced

to silver atom to form nanoparticles. This shows the effect of the lack of chemical linker between the surface and the nanoparticle.

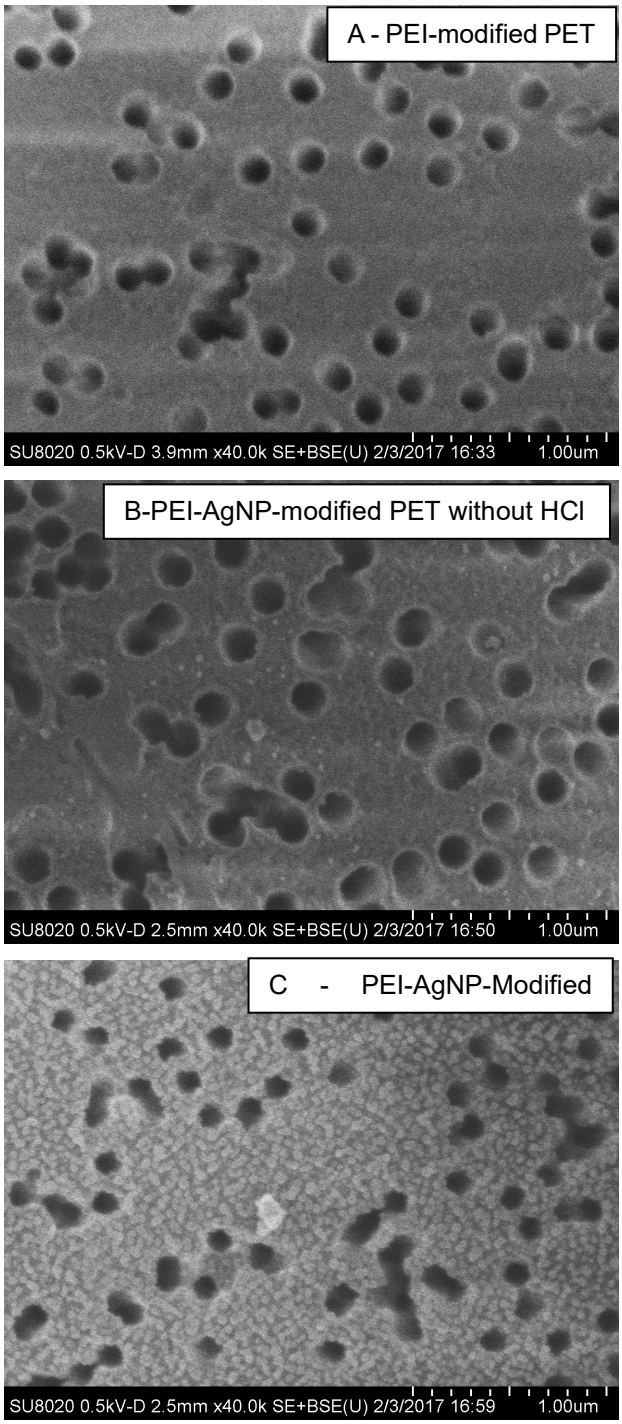


Figure 3.14: SEM images of unmodified PET membranes (A), PET-modified without HCl-activation (B) and PET-modified with HCl-activation (C) exposed to the reaction of AgNO3 and Na3C6H5OO7 at 90 degrees Celsius for 20 minutes.

The SEM micrographs in Figure 3.14 show that there were no silver nanoparticles chemisorbed on the surface of the unmodified PET membrane and that average pore sizes of the track etched membrane were in the region of 100 nm in size (A). The PET membrane (B) that was modified but not activated in

acidic media had negligible nanoparticles present on the surface. The SEM micrograph for the PET membrane (C) that was modified and activated in the acidic media shows full coverage of the surface by spherical silver nanoparticles of around 50 nm in size. The particles are larger in size than those sparsely shown on the PET membrane that was not acid activated. Thus, it can be inferred that acid activation of the triamine modified PET membrane provided the electrostatic attraction of the silver nanoparticles formed in the solution, compared to unmodified and non-acid activated membrane. Chemisorption of nanoparticles was enhanced on the surface of the membrane by activation of the membrane in acidic media.

3.2.2 Raman characterisation of membranes for Surface-Enhanced Raman Spectroscopy

The silver nanoparticles were coated on the surface of modified polyethene terephthalate (PET) membranes in order to be used as surface-enhanced Raman spectroscopy substrates to enhance the weak Raman signal with the aim of detecting acetaminophen molecules on the surface. The immobilisation of silver nanoparticles was done at different times, as that determined how much of the nanoparticles were deposited on the surface. The immobilisation was via the nitrogen of the amine that was used to modify the surface of PET membrane as indicated in Figure 3.4 (reaction mechanism). In Figure 3.15 are the Raman spectra of the silver-coated PET membrane and the uncoated PET membrane. The Raman spectra of silver-coated PET membrane still show Raman spectra polyethene terephthalate but not as prominent as for the uncoated PET membrane (CO-AgPET). The silver-coated PET membranes show that as the Ag nanoparticle immobilisation time was increased from 10 minutes to 30 minutes, the PET spectra was suppressed. This was found to be good for the detection of the analyte on the surface of the silver-coated PET membrane since there would not be interference from the PET membrane Raman spectra. The sample 30-AgPET was therefore chosen as a platform for detection of analytes of interest using surface-enhanced Raman spectroscopy. The sample 30-AgPET was chosen because the coated PET membrane showed the most negligible spectrum of PET membrane itself.

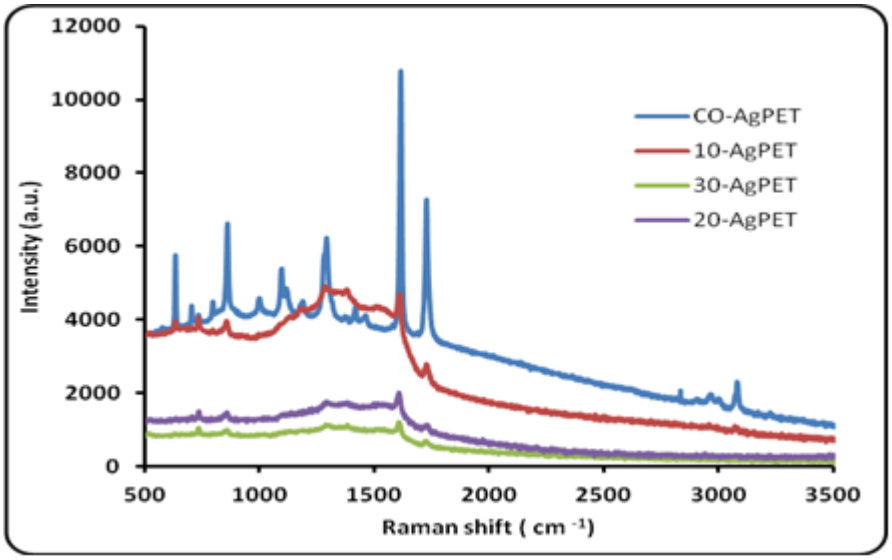


Figure 3.15: Raman spectra of silver-coated and uncoated PET membrane

3.3 CONCLUSION

The modification of track-etched membranes with triamine at ambient room conditions was successfully achieved via a wet chemistry method. The changes in colour of reduced silver nitrate solution from clear solution to pale yellow then orange clearly showed the formation of silver nanoparticles. This is also supported by the change in colour of the acid-activated amine-modified PET membrane that furthermore also changed colour with time as silver nanoparticles were synthesised. The changes in colour of the PET membranes showed that there was electrostatic attraction between the acid-activated modified membranes and the Ag nanoparticles. This was visible when comparing the unmodified PET against the acid-activated and the non-acid-activated PET membrane. It can therefore be concluded that the PET membranes were successfully modified and immobilised with silver nanoparticles via the nitrogen atom of the functionalised amine. These results are complemented by the XPS results that show the presence of nitrogen and silver on the surface of modified PET membrane. Also transmission electron microscopy (TEM) results confirm the successful deposition of Ag nanoparticles on the surface of the PET membrane using the DETA route.

4 DETECTION OF CONTAMINANTS IN SIMULATED WATER USING SERS

4.1 INTRODUCTION

There is a wide range of applications of surface-enhanced Raman spectroscopy (SERS) as an analytical technique in the fields of biotechnology (Boujday et al., 2015), food industry, warfare anti-terrorism, drug abuse (Craig *et al*., 2013) and environmental applications (Halvorson and Vikesland 2010), electrochemistry, surface and material science (Hering *et al*., 2008). The SERS technique has attracted many researchers in environmental pollutant analysis, with the main focus on detecting, identifying and quantifying very low concentrations of pollutants found either in air or water sources (Le Ru and Etchegion, 2008). The driving force behind research and development of SERS is the trace level analysis capability of the technique and cost effectiveness (Lucotti and Zerti, 2007). The technique also offers good practical utility (Li *et al*., 2014). SERS has several analytical advantages over other methods including ultra-sensitivity, selectivity and inherent molecular specificity (Boujday et al., 2015; Huh *et al*., 2009). Chemical analysis by SERS requires little or no sample preparation (Zhang *et al*., 2015), is convenient and cost effective for development of miniaturised equipment (Ma *et al*., 2015; Lucotti and Zerbi, 2007). SERS has an edge over infrared spectroscopy, as it can be directly applied in the aquatic environment with negligible background noise due to low polarisability index of water (Li *et al*., 2014). Although similar techniques such as fluorescence are already well established, the emerging SERS has attractive properties such that it can be used both in the near-infrared and the visible spectral region and does not require labelling the analyte of interest as is practised in the fluorescence technique (Cialla *et al*., 2012). The performance of SERS is based on its sensitivity, which depends on the surface property of SERS active materials (Botti *et al*., 2014) that can be tailored to suit the intended application (Péron et al*.,* 2009; Costa *et al*., 2006). It is envisioned that SERS could be used to simultaneously identify multiple pollutants in a sample reliably, rapidly and at lower cost (Halvorson and Vikesland, 2010). The SERS technique is flexible such that it can be applied in sequence with other separation techniques, such as nano filtration polymer membrane technology and chromatography (Muehlethaler *et al*., 2015) scanning probe microscopy and microfluidics (Cialla *et al*., 2012).

4.2 EXPERIMENTAL

The detection of a chosen pharmaceutical, acetaminophen using a silver-decorated track-etched polyethylene terephthalate membrane was carried out at different concentrations of acetaminophen in distilled water. The detection was carried out by dropping and drying a solution of acetaminophen on the surface of unmodified and silver-coated PET membrane and then the samples were placed under the probe of Enspectr R532 model Raman spectrometer. An exposure time of 600 ms, magnification of x10 were used with a 532 nm laser wavelength at the power of 20 MW.

4.2.1 Detection using silver-coated track-etched polyethene terephthalate membrane by surface-enhanced Raman spectroscopy

The Raman spectroscopy technique was used to detect the presence of acetaminophen on the surface of unmodified and silver-coated track-etched polyethene terephthalate membranes. In the study, it is expected that the silver nanoparticles on the surface would enhance the Raman signal, and therefore generate a more visible and readable spectrum of acetaminophen. For concentration studies, three concentrations with sample codes in brackets, of 15.1 mg/L (Aceta-100), 1.51 mg/L (Aceta-010) and 0.151 mg/L (Aceta-001) were prepared in 20 µL aliquots. A 20 µL of acetaminophen was dropped on the surface of a 1x 1 cm piece of the membrane and was left to dry under ambient room conditions for 24 hours. When the acetaminophen solution was dried, it was then placed on a platform of the Raman spectrometer for detection. The sample codes are described in Table 4.1.

Table 4.1: Experimental parameters and sample codes for detection of acetaminophen using 30-AgPET sample on surface-enhanced Raman spectroscopy (SERS)

	Volume	Time	Concentration	Sample code
Acetaminophen	20 µL	24 Hrs	15.10 mg/L	Aceta-100
	20 µL	24 Hrs	1.510 mg/L	Aceta-010
	20 µL	24 Hrs	0.151 mg/L	Aceta-001

4.2.2 Raman spectroscopy

Raman spectroscopy was carried out using a portable version of Enspectr R532 Raman spectrometer to study the surface chemistry of unmodified and modified track-etched polyethene terephthalate (PET) membrane samples. The spectrometer was also used for the surface-enhance Raman spectroscopy (SERS) application to detect analytes on the surface of unmodified and modified track-etched PET membranes besides the surface chemistry of silver-coated track-etched PET membrane. The Raman spectrometer is equipped with an internal laser of excitation wavelength 532 nm. The spectrometer is supplied with the objective lens, Olympus CX22 LED of magnification x10 and x40. The output power used was 20 mW. The exposure time was set at 600 ms with 20 frames. A manual locator was used to find the spot where to place the sample on the sample holder. Manual adjustments were properly made to get the right focus in order to get a focused spectrum. The sample preparation for SERS application was done by drop and dry method. An analyte solution was dropped on the surface PET membrane and left to dry at room temperature before characterising with the spectrometer.

4.3 RESULTS AND DISCUSSION

The results and discussion of the detection of acetaminophen on a modified surface of track-etched polyethene terephthalate (PET) membrane that was fabricated as outlined in the previous chapter are presented in this section. This section lays out the results and the applicability of surface-enhanced Raman spectroscopy using a silver-coated surface of PET membrane (10-AgPET, 20-AgPET and 30-AgPET samples (From Table 2.2)) for detection of acetaminophen. The focus is on whether the silver-coated track-etched PET membranes would be able to enhance a weak Raman signal resulting from

Raman scattering by acetaminophen molecules on the surface. The samples were characterised by Raman spectroscopy as described in Section 4.2.1. A discussion of the challenges encountered to detect acetaminophen on the surface of both unmodified track-etched PET (Co-AgPET) and silver-coated track-etched PET membrane (10-AgPET, 20-AgPET and 30-AgPET samples) using the Raman spectrometer is given.

The moieties of acetaminophen have specific vibration modes that produce a Raman spectrum specific to acetaminophen (Kauffman et al., 2008). The chemical structure of acetaminophen is shown in Figure 4.1.

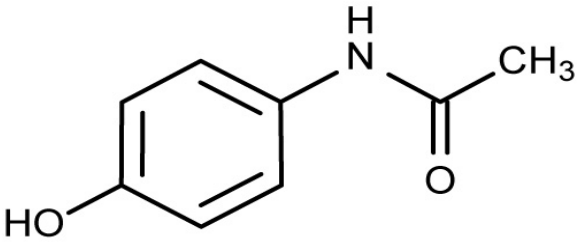


Figure 4.1: Chemical structure of acetaminophen

Acetaminophen has functional groups which have peaks in its Raman spectrum, which are also common to other chemicals; the functional groups are phenyl, amide, carbonyl and hydroxyl. Although acetaminophen has similar peaks to common functional groups, its overall Raman spectrum is specific to it only, making it a fingerprint signature. The typical acetaminophen peaks identified in its spectrum are outlined in Table 4.2 (Kauffman et al., 2008).

Table 4.2: Peaks in Raman spectrum of acetaminophen.

Proposed bond	Spectral peak cm^{-1}
C-O	854, 862, 1172
C-N	1234, 1245, 1281
C=C	1557, 1565, 1576
C=C	1611, 1615, 1623
C=O	1646, 1650

The peaks of the Raman spectrum in Table 4.2 were used to ascertain the presence of acetaminophen on the prepared surfaces of unmodified track-etched PET membrane (Co-AgPET), silver-coated track-etched PET membrane (10-AgPET, 20-AgPET and 30-AgPET samples) and a control surface made of silver and titanium oxide on a glass support (Quartz).

4.3.1 Preparation for Surface-enhanced Raman Spectroscopy platform

The silver nanoparticles were coated on the surface of modified track-etched polyethene terephthalate (PET) membranes as surface-enhanced Raman spectroscopy active materials to intensify the weak Raman scattering signal with an aim of detecting acetaminophen molecules on the surface. The

immobilisation of silver nanoparticles was done at different times in order to find the best conditions that would suppress the polyethene terephthalate Raman signal.

Figure 4.2 shows the Raman spectra of the silver-coated track-etched PET membranes (10-AgPET, 20-AgPET, 30-AgPET) and the unmodified track-etched PET membrane (Co-AgPET) as a baseline (control sample).

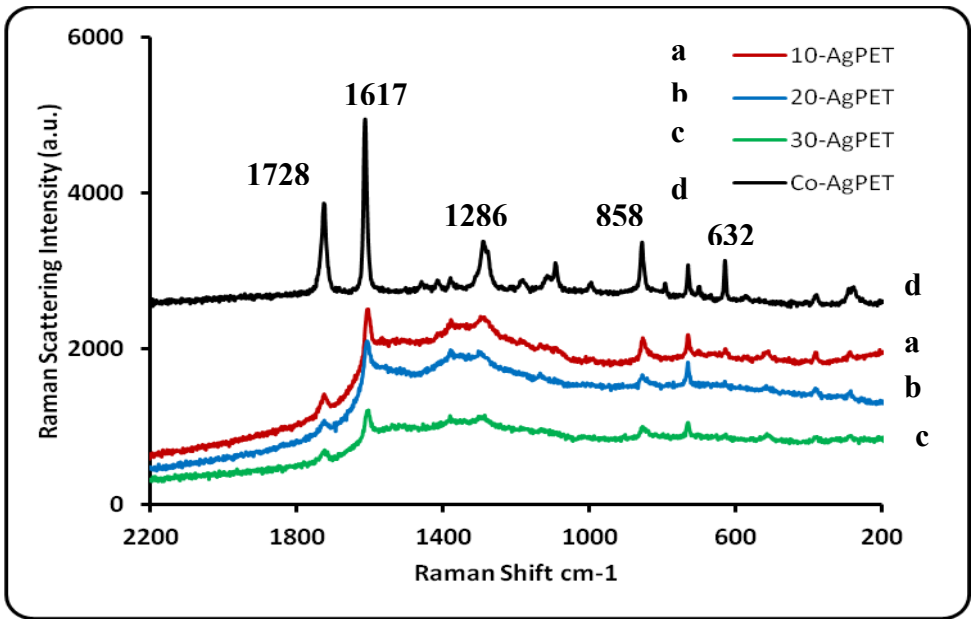


Figure 4.2: Raman spectra of silver-coated track-etched PET membranes at reaction times (a) 10 minutes (10-AgPET), (b) 20 minutes (20-AgPET), (c) 30 minutes (30-AgPET) and unmodified PET membrane (d) control (CO-AgPET) prepared at 90°C using 2 mL of 1% trisodium citrate

The results for Raman spectra in Figure 4.2 show that as the time of silver nanoparticle immobilisation increased, the intensity of the peaks relating to the PET membrane were reduced with the lowest signal at 30 minutes as shown by the spectrum of 30-AgPET in Figure 4.2. The Raman spectra of silver-coated track-etched PET membranes (10-AgPET, 20-AgPET, 30-AgPET) show suppressed peaks relating to the polyethene terephthalate membrane and these peaks were not as prominent as for the unmodified track-etched PET membrane (Co-AgPET). The Raman spectra peaks of silver-coated track-etched PET membranes show that as the silver nanoparticle immobilisation time was increased from 10 minutes to 30 minutes, the PET peaks' intensities were reduced and suppressed. The reduction in the PET peaks' intensities follows the trend of time of silver nanoparticles immobilisation on the surface of amine-modified track-etched PET membranes. The trend observed showed that as time of reduction reaction increased, so did the size of silver nanoparticles immobilised on the surface of PET. The silver-coated track-etched PET membrane coded 30-AgPET was therefore chosen as the most suitable platform for detection of acetaminophen using surface-enhanced Raman spectroscopy. The Raman spectrum of sample 30-AgPET was chosen as baseline because the silver-coated track-etched PET

membrane showed the minimum peak intensities of the PET membrane itself. The cut-off point of immobilising silver nanoparticles on modified track-etched PET membrane was set at 30 minutes because the size of silver nanoparticles of sample 30-AgPET fell within the average range of 58 nm as stated by Taurozzi and Tarabara, 2007.

4.3.2 Detection of acetaminophen using fabricated silver-coated track-etched polyethene terephthalate membrane.

Similar to Fourier transform infrared spectrum, a Raman spectrum comprises wavelength distribution of peaks equivalent in character to molecular vibrations specific to the analyte being characterised (Ferraro, 2003). Surface-enhanced Raman spectroscopy (SERS), as an advanced Raman spectroscopy technique, was used to detect acetaminophen using silver nanoparticles as SERS active materials to enhance the Raman signal. The detection of the molecules of acetaminophen on the silver-coated track-etched polyethene terephthalate membrane was carried at ambient conditions. The Raman spectra of acetaminophen detected on the surface of silver-coated track-etched polyethene terephthalate (PET) membrane (30-AgPET), unmodified track-etched PET (Co-AgPET) and Quartz, a silver-coated glass surface (non-porous), are shown in Figure 4.3. A solution of 20 µL of 1.51 mg/L aqueous acetaminophen was dropped and dried on the surfaces of samples.

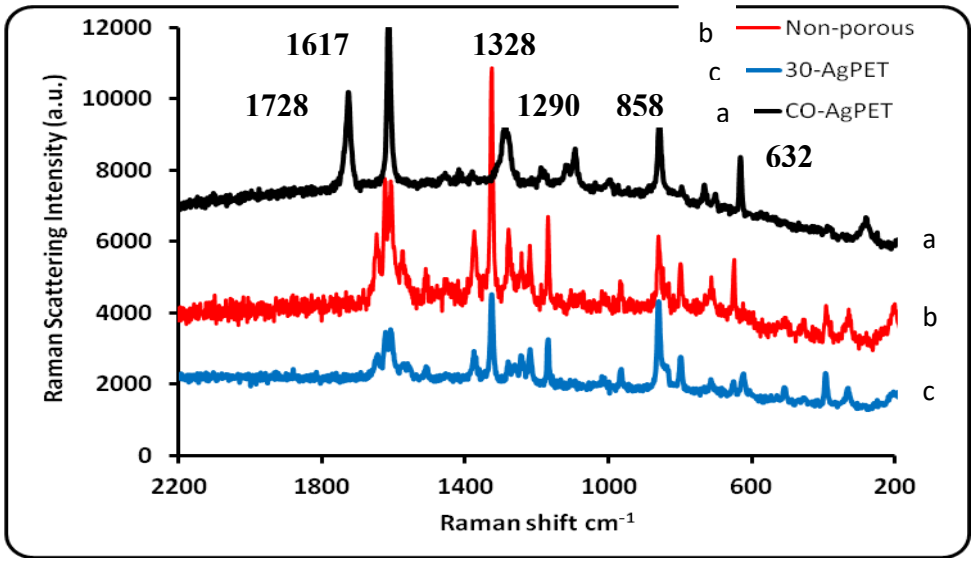


Figure 4.3: Raman spectra of 1.51 mg/L of acetaminophen on the surface of (a) Co-AgPET (unmodified track-etched PET) membrane (b) quartz (Non-porous) membrane and (c) 30-AgPET (silver-coated track-etched PET membrane)

Figure 4.3 shows that the peak intensities are higher for the sample coded Quartz (a non-porous silver-coated glass) than that of 30-AgPET, the track-etched silver-coated track-etched PET membrane. In the case of the control sample, Co-AgPET, which is unmodified track-etched PET membrane, no characteristic peaks of acetaminophen could be observed among the overall prominent peaks of polyethene terephthalate membrane. This could be attributed to the lack of Ag nanoparticles to

enhance the acetaminophen Raman fingerprint on the surface of sample Co-AgPET membrane. Therefore it is inferred that the lack of silver nanoparticles is the main cause of the lack of acetaminophen peaks for the sample Co-AgPET. When comparing the Raman signal intensity of acetaminophen on sample Quartz (a non-porous SERS surface) against 30-AgPET (a track-etched silver-coated track-etched PET membrane), most of the vibrational bands are rather weak. The limited SERS intensity and the low spectral intensities could be ascribed to the loss of some molecules of acetaminophen that leach through the track etched PET pores, indicating that a smaller pore size could be more suitable to prevent loss of the analyte.

4.3.3 Concentration studies for detection of acetaminophen

Concentration studies were carried out using a silver-coated track-etched polyethene terephthalate (PET) membrane sample 30-AgPET for the detection of different concentrations of acetaminophen as described in Section 4.2.1. Acetaminophen solution was prepared using distilled water. The following concentrations were prepared 15.10 mg/L, 1.51 mg/L and 0.151 mg/L which were coded Acet-100, Acet-010 and Acet-001 respectively. Acetaminophen solution of volume 20 µL for each concentration was dropped and dried on the surface of 30-AgPET. The Raman scattering intensity varied with a change in the concentration of the acetaminophen as shown in Figure 4.4.

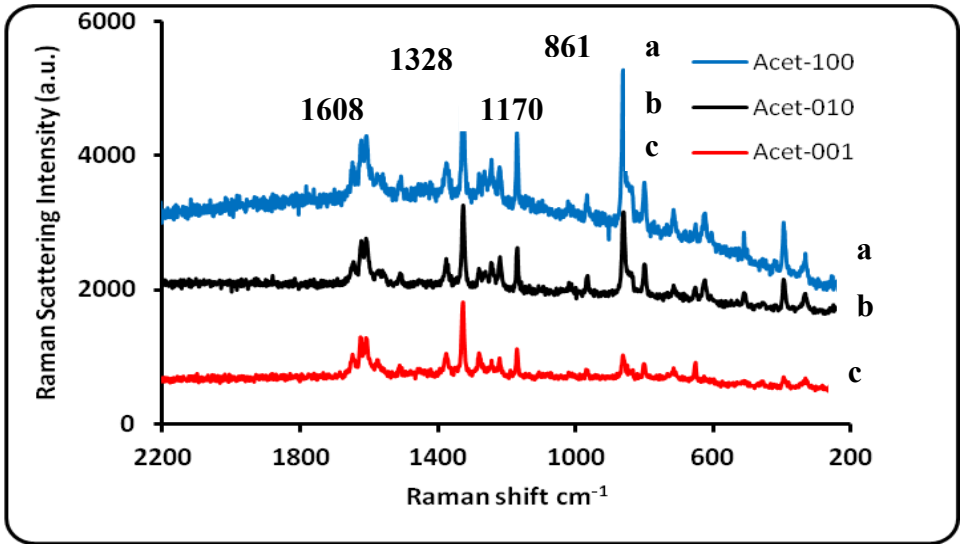


Figure 4.4: Variation in Raman spectra intensity of different concentrations of acetaminophen in aqueous media (a) 15.1 mg/L, (b) 1.51 mg/L and (c) 0.151 mg/L.

Figure 4.4 shows that the intensity of the Raman peaks increased with an increase in acetaminophen concentration. The trend agrees with what was reviewed in the literature that Raman peak intensity is a function of the concentration of analyte (Taurozzi and Tarabara, 2007). Sample Aceta-100, which is 15.1 mg/L has its acetaminophen peak intensity greater than those of lower concentration (Aceta-010 and Aceta-001), which were 10 and 100 times diluted, respectively. The changes in the intensity of the peaks of the acetaminophen spectrum are consistently changing with change in concentration.

Theoretically, when there is a higher number of molecules on the surface-enhanced Raman spectroscopy (SERS) surface hot sites, there is a greater chance of observing medium to strong Raman signals (Strachan *et al*., 2007). This is also shown in peaks of the spectra in Figure 4.4. When more acetaminophen molecules covered the surface of silver-coated track-etched PET membrane, the possibility of observing a strong to medium Raman signal was greater. The SERS effect is provided by the localised surface plasmon of silver nanoparticles. The higher Raman scattering intensity could be as a result of SERS contributions from both electromagnetic effects and change transfer (chemical effects) arising from adsorption of acetaminophen molecules on the silver nanoparticles. There is a greater probability for the acetaminophen to be adsorbed on the silver nano particles if it exists in high concentration on the surface.

4.3.4 Application of spectral peaks for quantification

Theoretically, quantification of an analyte in a sample is possible with Raman spectroscopy since the Raman scattering is proportional to the concentration of the analyte. The intensity of Raman scattering of a particular vibration mode is directly proportional to vibrating moieties' concentration (Strachan et al., 2007). Raman spectra analysis could be used to extract information from the peak area, or use the ratio of peak area to quantify the analyte. This could be possible only if spectral peaks do not overlap, and all analyte samples are exposed to the same conditions so as to be affected equally by undetected interferents. The challenge with the peaks is that the proportionality does not seem to be the same for all peaks, that is, the peak's increase with increasing concentration is not the same for all spectrum peaks. This could present a challenge if the peak area is to be used for a calibration curve, which is to be used for calculation of concentration as is done with other techniques such as gas chromatography. The detection and quantification of acetaminophen on the surface of silver-coated track-etched polyethene terephthalate (PET) or unmodified track-etched PET was thus faced with some challenges. The Raman spectrometer used for detection had limited options, for instance it is limited to a laser range of 532 nm, and a lack of software to calculate peak heights and area.

Theoretically, quantification of an analyte in a sample is possible with Raman spectroscopy since the Raman scattering is proportional to the concentration of the analyte. The intensity of Raman scattering of a particular vibration mode is directly proportional to vibrating moieties' concentration (Strachan et al., 2007). Raman spectra analysis could be used to extract information from the peak height or area, and/or use the ratio of peak height or area to quantify the analyte. This could be possible only if spectral peaks do not overlap, and all analyte samples are exposed to the same conditions so as to be affected equally by undetected interferents. In reference to Figure 4.4 showing Raman spectra of concentrations 15.10 mg/L, 1.51 mg/L and 0.151 mg/L represented as Acet-100, Acet-010 and Acet-001 respectively, Figure 4.5 shows the trend in specific Raman intensity peak heights (861, 1170, 1328 and 1608 cm^{-1}) relative to the concentration of acetaminophen.

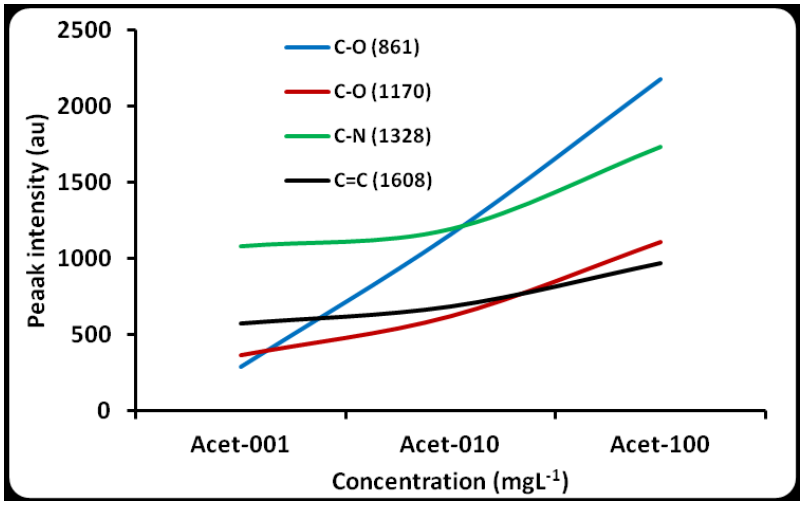


Figure 4.5: Graphical presentation of relationship between concentration of acetaminophen and trends in Raman intensity peak height at specific bond vibrations (a) C-O (861), (b) C-O (1170), (c) C-N (1328) and C=C (1608)

The general trend in Figure 4.5 shows that as the concentration increased so did the peak intensity height. The difference in the trends is observed when comparing the rate of change amongst the specific bonds. For instance the rate of change of C=C (1608) and C-0 (1170) are not consistently correlated to an increasing concentration, but rates of change for C-O (1170) and C-N (1328) had a similar trend. The peak intensity trend for the C-O (861) vibration showed a linear response. The linearity of peak intensity of the C-O bond versus the concentration had the best correlation of the two factors. The other bond vibrations C-O (1170), C-N (1328) and C=C (1608) showed no linearity and if extrapolated towards zero concentration they crossed the peak intensity axis instead of the concentration axis. The linear correlation line of C-O (861) when extrapolated would give a limit of detection of approximately 0.0755 mg/L. The challenge with the peak intensity heights is that the proportionality does not seem to be the same for all peak intensity heights, that is, the peak intensity increases were not the same for all spectrum peaks. The finding has not been reported anywhere else in literature and therefore this is the first observation to have been made by this study.

4.4 CONCLUSION

The modification of track-etched membranes with triamine at ambient room conditions was successfully achieved via a wet chemistry method. The modification of the surface of polyethene terephthalate membrane was successfully confirmed by Fourier transform infrared and X-ray photoelectron spectroscopy. The modification was also visually noted by the change of colour of the amine-modified PET membrane from white to pale yellow then to silver colour as a time of immobilisation was extended from 10 minutes to 30 minutes. The Raman spectroscopy characterisation of the silver-coated PET membrane showed that the more the silver nanoparticles were deposited on the surface of amine-modified PET membrane, the more nanoparticles obscured detection of the membrane vibrations. This resulted in negligible PET peaks in the Raman spectrum, which is a good development for a platform on which analytes of interest could be detected by surface-enhanced Raman spectroscopy. The silver-

coated PET membrane fabricated after 30 minutes of nanoparticle immobilisation successfully showed spectra of acetaminophen when dropped and dried on the surface. Whilst 10 minutes silver-coated PET membrane failed to suppress the PET signal, therefore, it could not be used to distinguish spectra of the PET from that of the analyte (acetaminophen). The spectrum of acetaminophen on a surface of the non-porous quartz surface was much more enhanced than that on the silver-coated track-etched PET membranes. This could be as a result of capturing of fewer molecules of acetaminophen on the surface of track-etched PET membrane, as they could easily have passed through the rather large pores. Therefore it is concluded that the surface of polyethene terephthalate was successfully modified with diethylenetriamine and immobilised with silver nanoparticles for detection of acetaminophen by surface-enhanced Raman spectroscopy, but that problems could arise due to the low signal intensity if analyte is lost or not upconcentrated sufficiently. Moreover, the study showed that not all Raman peaks could be used for quantification.

5 COMPARISON OF ANALYTICAL METHODS AND VALIDATION OF THE DEVELOPED METHOD

5.1 INTRODUCTION

Anthropogenic water pollution has become an increasingly serious environmental problem (Li et al., 2014). This has arisen due to the exponential growth of the human population, thereby putting more stress on already scarce clean water sources (Mokhena et al., 2015). South Africa is one of the countries with a high scarcity of fresh water arising from highly variable and spatially distributed rainfall. Because of this, there is a growing interest worldwide to reuse wastewater so as to address some of the challenges associated with clean water shortages (Mulamattathil et al., 2014). The reuse of wastewater could augment conventional water supplies through improving the quality of unconventional water sources (Qu et al., 2016). Wastewater reuse affects the quality of water if proper treatment procedures are not followed. The use of recycled wastewater for consumption exposes users to high levels of persistent organic pollutants such as personal care products and pharmaceuticals (Stackelberg et al., 2004). Pharmaceuticals and personal care products have been detected at low concentrations in wastewater treatment plant effluent, surface water, drinking water and groundwater (Pavlović et al., 2013; Ratola et al., 2012). These persistent organic pollutants find their way into water systems from wastewater treatment plant effluents due to ineffective treatment protocols and due to improper disposal of pharmaceuticals and personal care products (Ratola et al., 2012).

Pharmaceuticals are human and veterinary medicinal compounds that have a specific activity in the body (Klavarioti et al., 2009). Pharmaceuticals are physically and chemically dynamic as they vary widely in molecular weight, structure, shape and their specific activities, which are influenced by functional groups (Nghiem et al., 2005). They are polar molecules and their properties vary with solution pH and temperature in aqueous environments. Some pharmaceuticals are lipophilic and moderately soluble in water. Others can modify their chemical structure when subjected to metabolic reactions (Rivera-Utrilla et al., 2013). Pharmaceuticals are discharged into the aquatic environment by the disposal of expired pharmaceuticals, inadequate treatment of wastewater treatment plants' effluents and leachate from pit latrines in developing countries (Wood et al., 2015; Schaider et al., 2014). These pharmaceuticals such as acetaminophen and sulfamethoxazole, have been detected in rivers, lakes and groundwater in European and Asian countries, wastewater treatment plant effluents and drinking water, which means that they are not completely removed and mineralised by current treatment methods (Pinhancos et al., 2011). For instance, ibuprofen and carbamazepine are stable during photo-degradation treatment. Although recently, there are increased numbers of published reports on pharmaceuticals in the aquatic environment, very little research has been carried out in South Africa to detect and identify pharmaceuticals in water sources. Pharmaceuticals such as antiretroviral compounds used for HIV treatment and other varieties of pharmaceuticals have been detected in water sources across South Africa (Wood et al., 2015). The pharmaceuticals may have potentially adverse effects on humans and the ecosystem (Schaider et al., 2014; Nikolaou et al., 2007). The various adverse effects include feminisation or masculinisation of aquatic organisms due to exposure to hormones as well as the development of microbial resistance to antibiotics (Agunbiade and Moodley, 2016). The challenge with the

presence of many different pharmaceuticals that exist in very low concentrations in the aquatic environment is that they require extensive sample preparation to be detected and identified (Tijani et. al., 2013), each compound requires a specific analytical process in order for the compound to be identified and quantified and also validate the results. In this section the various analytical procedures for identification and quantification of selected compounds on the SERS platform will be compared to the GC-MS and HPLC-MS systems.

5.2 EXPERIMENTAL APPARATUS

5.2.1 Preparation of standard solutions

The stock solution of the standards (Table 5.1) was prepared at 1000 µg/mL in methanol and stored at 4 ^{0}C in glass vessels until used. The preparation of the working solutions from the stocks was achieved by diluting specific concentration into methanol. The stock solutions were allowed to warm up to room temperature and mixed with a mixer before use. The working solutions of the analyte standard were made as 5, 2, 1, 0.5, 0.2, 0.1, 0.05 µg/mL**.**

5.2.1.1 Sample preparation with solid phase extraction (SPE)

a. Materials

The SPE device VacMaster (Biotage® VacMaster™ 20 Sample Processing Station) was used for sample concentration (solid phase extraction of the analyte sample). The analyte sample was preconcentrated with Strata-X 33µm polymeric C18 reverse phase columns (500 mg/6 mL) which were obtained from Phenomenex (Copenhagen, Denmark). The Reacti-Vap Evaporation unit (Thermo Scientific, Vantaa Finland) was used for evaporation of the extracts under N_2 gas. All waters used were purified with a Direct-Q UV Millipore water purification system (Millipore S.A., Molsheim, France).

b. Procedure

2 L Milli-Q water spiked with 4 ppm of the analyte (Acetaminophen) was pre-concentrated by running through the SPE cartridge (2 cartridges for 1 L of sample). Prior to the extraction process, the SPE cartridges (C18 columns) were preconditioned with 6 mL absolute methanol (HPLC grade) and 6 mL Milli-Q water was also used to flush the cartridges thereafter. The analyte sample was run through the cartridges at a slow rate. Thereafter, the sorbent materials (SPE cartridges) were left to dry for 12 hours. Extraction from the sorbent materials was then carried out by running 6 mL methanol slowly through each one. The eluates were collected in test tubes and separately evaporated under nitrogen with mild heating (40°C) to dryness, followed by dissolution with 2 mL methanol with agitation. The sample volume from the C_{18} columns was 2 mL. The final sample volume of 250 µL was separated from the pre-concentrated analytical sample for the analysis.

c. Drug sample and chemical reagents

Table 5.1: Drug sample and chemical reagents

Chemical and Reagents	Supplier	% Purity
Diclofenac Sodium Salt	Sigma Aldrich, South Africa	Analytical grade
Caffeine	Sigma Aldrich, South Africa	Analytical grade
Ibuprofen Sodium Salt	Sigma Aldrich, South Africa	98
Acetaminophen	Sigma Aldrich, South Africa	98-102
Methanol HPLC grade	Sigma Aldrich, South Africa	99.9
Absolute Ethanol	Sigma Aldrich, South Africa	99.9
HCl analytical grade	Science World, South Africa	36.46
Silver Nitrate	Alfa Aesar, Germany	Analytical grade
Trisodium Citrate	Alfa Aesar, Germany	99
Diethylenetriamine	Alfa Aesar, Germany	98
Derivatisisng agent(BSTFA)	Sigma Aldrich, South Africa	
Pyridine	Sigma Aldrich, South Africa	

5.2.1.2 Sample Preparation

a. Diclofenac, Ibuprofen and Acetaminophen were each dissolved in a solution of 50:50 Methanol and Distilled Water and then were mixed to make a 1000 ppm solution of each drug. Each xenobiotic concentration was prepared in triplicate for repeatability. Concentrations of 5, 2.5, 1, 0.5, 0.25, 0.1, 0.05, 0.025, 0.01 ppm were prepared for the calibration curves by serial dilution of each drug. Triplicates of samples were prepared by serial dilution to make up concentrations of 0.75, 0.3, 1.5 ppm for validation.
b. Pure samples of Ibuprofen with concentrations of 5, 2, 1, 0.5, 0.2, 0.1, 0.02, 0.001 ppm were prepared by serial dilution. 5 micro litres of Ibuprofen was placed on the modified PET-Ag membrane and left overnight to dry at room temperature.

5.2.2 Determination of LOD and LOQ

Limits of detection (LOD) and quantification (LOQ) were determined on the basis of the standard deviation of blank-sample responses and the slope of the calibration curve for each analyte. The instrumental limit of detection (LOD) and limit of quantification (LOQ) was determined for each compound by injecting standards at 0.02, 0.05, 0.1, 0.5, 1, 2.5, 5, 10 and 25 mg/L on the SERS platform, and compared to the GC-MS and HPLC-MS systems. Linear Regression in Excel, Detection Limits and ICH Guidelines was used to calculate the statistical data for LOD & LOQ. The three sigma rule relates that the data falls within a normal distribution. The LOD is 3 times signal to noise (S/N) whereas the LOQ is 10 times S/N LOQ.

5.2.3 Analytical techniques

5.2.3.1 GC-MS Analysis

5 µL of each concentration of the standard samples and the samples was pipetted into tubes to dry in a Cetrivap Concentrator overnight at 350C, 15 bars. Once dried the samples were derivatised for 1 hr at 80°C in the oven using 30 µL BSTFA in 100 µL Pyridine. Polar compounds are thermally unstable and hence require derivatisation so that they have properties suitable for GC analysis.

Chromatographic Separations

Thereafter, 1 µL of the sample was injected on a Thermo TSQ 8000 triple quadrupole MS operated in a selected reaction monitoring (SRM) mode. Separation of the pharmaceuticals was performed with a Rxi ® 1310 gas chromatograph coupled to a non-polar Rxi®-5Sil MS w/integra-Guard (15 m, 0.25 mm ID, 0.25 µm film thickness) capillary column. Initial oven temperature was 100°C, held for 3 min to a final temperature of 200°C at 12°C/min, and held for 5 mins. The injector temperature was maintained at 250°C, injection was splitless and carrier gas Helium was used at 1 mL/min. The transfer line and ionisation source temperatures were set at 280°C and 250°C respectively and emission current of 50 µA was used with Argon collision.

5.2.3.2 LC-MS Analysis

A Waters Acquity ultra performance liquid chromatography (UPLC) coupled to a Xevo TQ-MS mass spectrometer (MS/MS) (Waters, Milford, MA, USA) was used for high-resolution UPLC-MS/MS analysis. Separation of the pharmaceuticals was achieved on an Aquity UPLC BEH C18 (2.1 x 100 mm; 1.7 µm particle size) column at 40 ˚C and a flow rate of 0.35 mL/min. Data was acquired with multiple reaction monitoring (MRM) using electrospray positive ionisation. The operating parameters used were as follows: capillary voltage, 3.5 V; cone voltage range, 10-35 V; collision energy range, 5-40 eV; source temperature, 140 ˚C; desolvation temperature, 400 ˚C; desolvation gas, 800 L/h and cone gas, 50 L/h. An injection volume of 5 µL was used and the mobile phase consisted of water acidified with 0.1% formic acid (A) and acetonitrile acidified with 0.1% formic acid (B).

5.2.3.3 SERS Analysis

Samples were also analysed using Raman spectroscopy. Analysis of unmodified, or modified PET membrane samples and detection of Ibuprofen analytes was carried out using Enspectr R532 Raman spectrometer to study the surface chemistry. The Raman spectrometer is equipped with an internal laser of excitation wavelength 532 nm. The spectrometer contains an objective lens, Olympus CX22 LED of magnification x10 and x40. The samples were spotted with 10x and 40x objectives. The exposure time was set at 600 ms with 20 frames. A locator was used to find the spot where to place the sample on the sample holder. The Raman spectrometer was manually adjusted to get the right focus and optimised for the experiment.

5.3 RESULTS AND DISCUSSION

5.3.1 Recovery studies for standard analytes using Solid Phase Extraction (SPE)

The efficiency of the quantitative recovery of the standard analyte (Acetaminophen) using the SPE method was evaluated using the data from the recovery experiment. The analyte was recovered from solution of 4 mg/L analyte standard in Milli-Q water. The relative percentage recovery of the analyte is given in Table 5.2.

Table 5.2: Mean recovery percentage for Acetaminophen by SPE method

Standards Analytes	Expected concentration (mg/L)	Measured concentration (mg/L)	Average recovery (%)
Acetaminophen	4	3.8	95.25

5.3.2 Comparison of analytical techniques

5.3.2.1 Liquid Chromatography-Mass Spectroscopy (LC-MS)

Below are representative chromatograms with each retention time window of the standard mixture of the pharmaceutical compounds which were analysed using MRM in the electronspray positive ionisation illustrated in Figure 5.1. LC-MS analysis involves ionisation of the analyte and identification by the Xevo TQ-MS, quadrupole mass analyser. The process involves the bombardment of the analytes with H^+ ions to produce parents and daughter with a +1 charge, which are then separated by the MS.

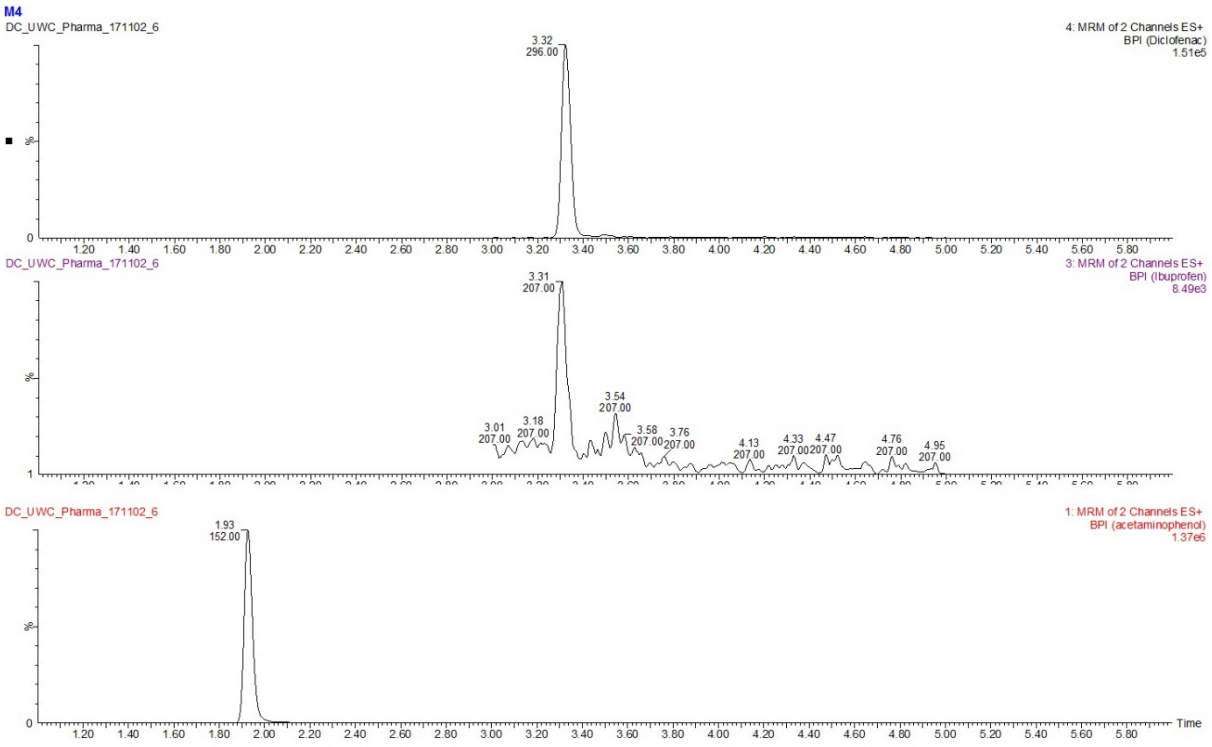


Figure 5.1: A chromatogram of a standard mixture for target compounds (diclofenac, ibuprofen, caffeine, acetaminophen) analysed by a positive ionisation mode

In this study the results show that acetaminophen has a lower affinity for the stationary phase compared to the rest of the compounds followed by caffeine >Ibuprofen 1>diclofenac1. This is due to its high pK^a value of 9.5 which is high compared to the other pharmaceuticals. It is worth noting that the acetonitrile mobile phase also has a high pK^a value of 25 and hence acetaminophen has a higher affinity towards the mobile phase than the stationary phase. In the analysis caffeine was expected to come last but was eluted second due to the fact that it has a small molecular mass and hence was eluted after acetaminophen. Besides, caffeine has no hydrogen atoms, it is non-polar compared to diclofenac and ibuprofen and hence dissolves easily in acetonitrile. The elution proceeds with ibuprofen with a molecular mass of 206.3 g/mol and lastly diclofenac 318.1 g/mol. Both diclofenac acid and ibuprofen are weak acids and hence are slightly polar and do not ionise well owing to their low interaction with acetonitrile. Also ibuprofen only has O atom as an electron withdrawing group which is further from the benzene ring hence the H-atom is easily ionised compared to diclofenac which has a N-atom a which is close to the benzene ring making the H atom less accessible for ionisation.

From the standards samples, calibration curves for each analysed drug were plotted and a line of best fit was obtained. The correlation coefficient r^2 was evaluated to determine whether the analytical tool would have a linear response to the analyte under study.

In 2019 a study was performed for ibuprofen, acetaminophen and diclofenac and each pharmaceutical compound was analysed using LC-MS. The sample standards for the experiment were mixed together to obtain the final concentrations which were used to obtain the calibration curves. This may have resulted in errors and inaccuracies in sample quantification by the instrument as there were probably interferences by the other drugs hence the repetition of the study. In particular, the Ibuprofen 1 calibration curve shown below in Figure 5.2 indicates a very low line of regression of 0.824517.

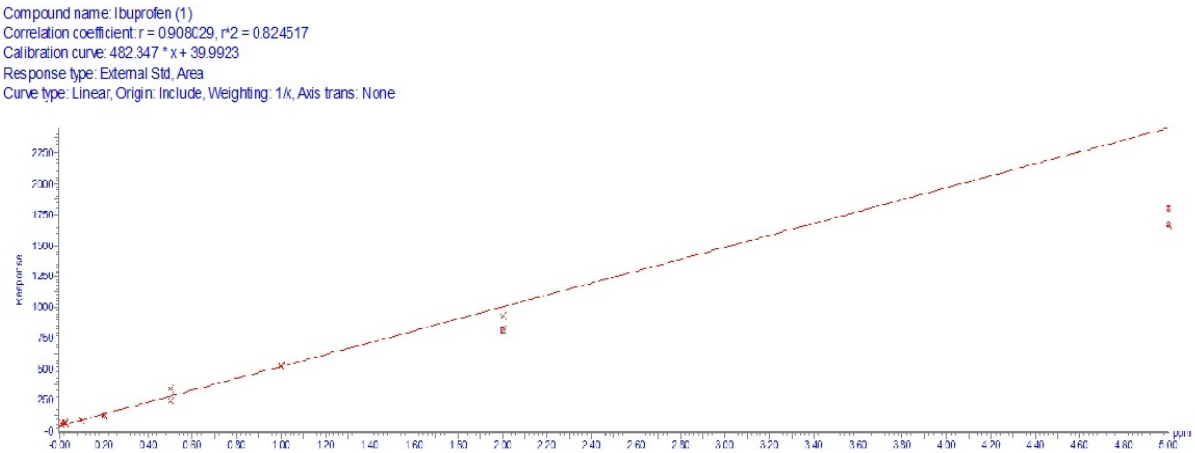


Figure 5.2: Calibration curve for Ibuprofen 1

The LC-MS calibration curves for ibuprofen, acetaminophen and diclofenac are shown below.

Coefficient of Determination: R^2 = 0.948374
Calibration curve: 260.802 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None

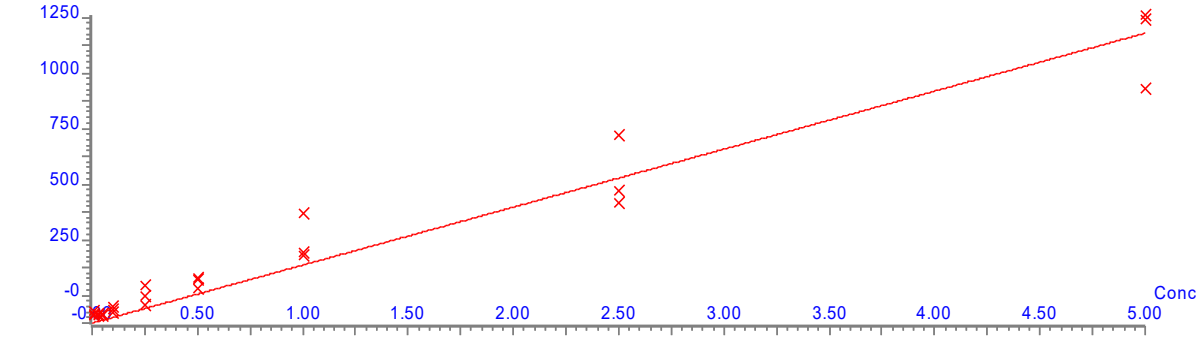


Figure 5.3: Calibration curve for Ibuprofen 1

Compound name: Ibuprofen pos
Coefficient of Determination: R^2 = 0.948374
Calibration curve: 260.802 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None

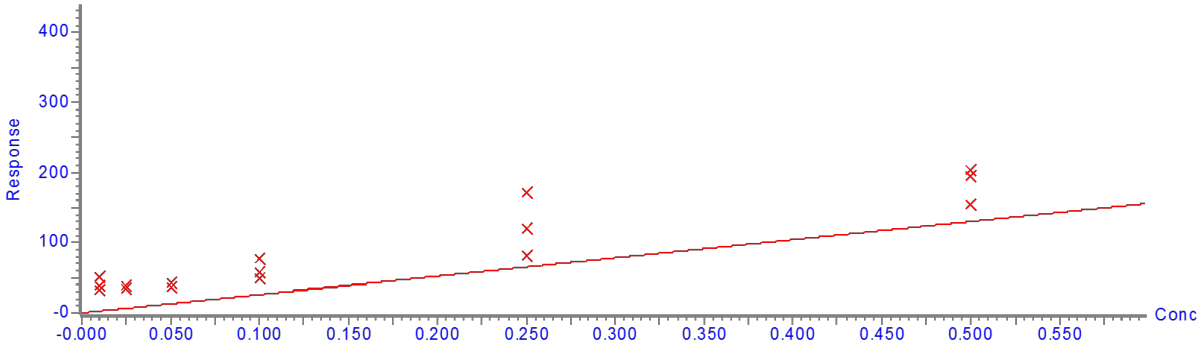


Figure 5.4: Calibration curve for Ibuprofen pos

Compound name: Ibuprofen pos
Coefficient of Determination: R^2 = 0.989834
Calibration curve: 272.961 * x
Response type: External Std, Area
Curve type: Linear, Origin: Force, Weighting: Null, Axis trans: None

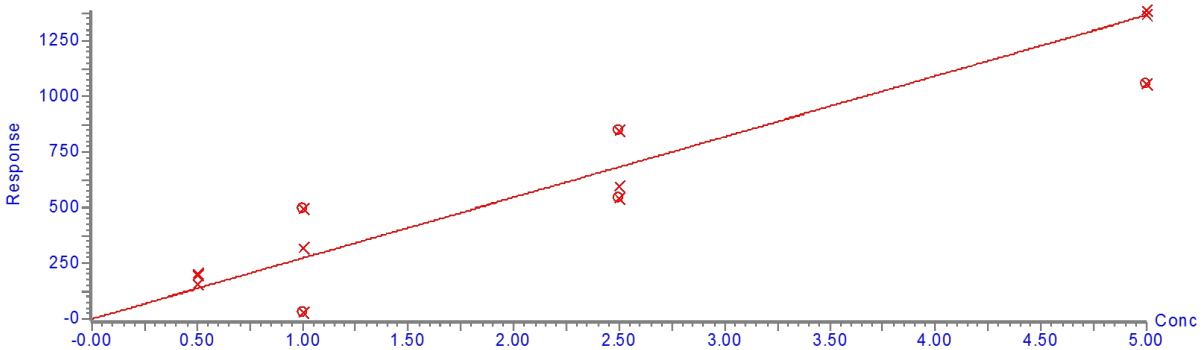


Figure 5.5: Calibration curve for Ibuprofen pos (0.5 ppm and above)

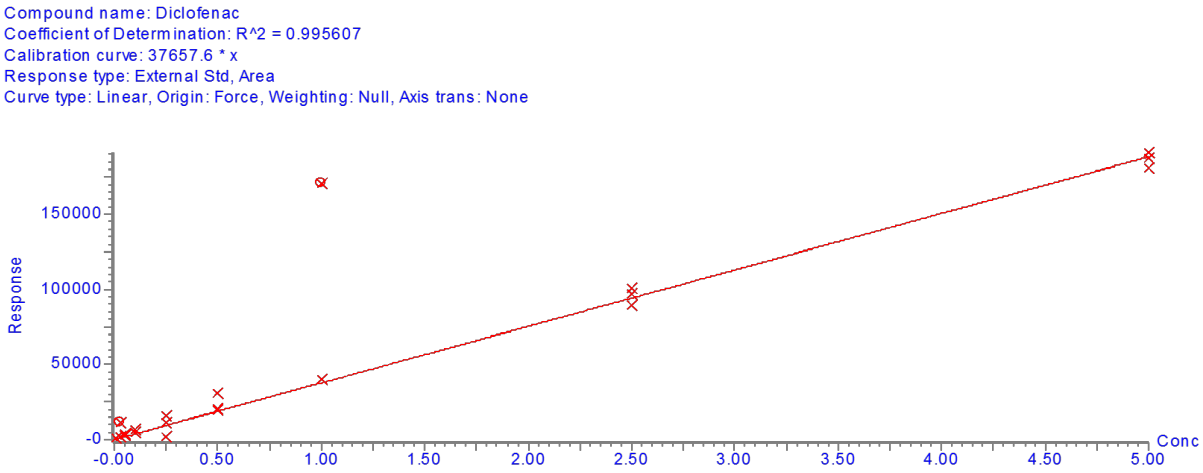


Figure 5.6: Calibration curve for Diclofenac

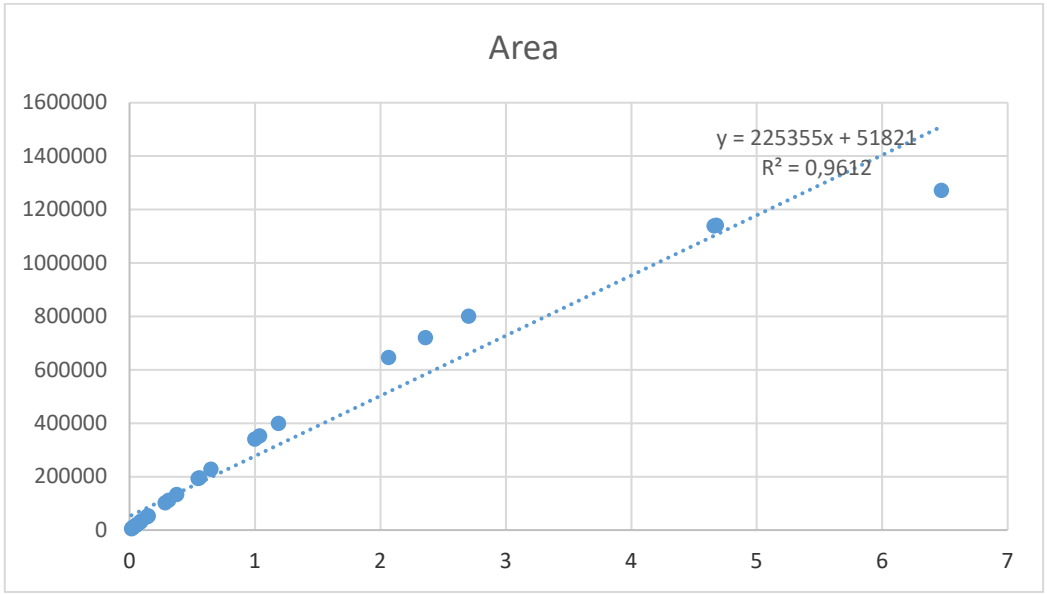


Figure 5.7: Calibration curve for Acetaminophen

Table 5.3: Linearity (regression coefficient), detection and quantification limits of detection (LOD, LOQ) of the LC-MS method (2019)

Compound	Regression line	LOD (ppm)	LOQ (ppm)
Acetaminophen	0.981586	0.747445358	2.264985934
Ibuprofen 1	0.989834	1.45565E-06	4.41106E-06
Diclofenac 1	0.995607	1.41504E-06	4.28801E-06

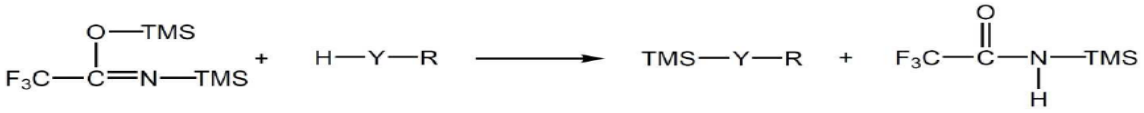
The above results show that LCMS is a good tool for analysing pharmaceuticals as it is repeatable and reliable. Ibuprofen correlation coefficient is close to 1 showing that it can only be analysed at high concentrations, and hence a positive relationship. These results were obtained at a 95% confidence interval.

The standard deviation of the obtained results was 0.0056 showing that the analyte concentration is close to the mean, and hence LC-MS is very sensitive and repeatable. The relative standard deviation criteria for

acceptance is >2% and in this study the value was 0.76% and hence showing that the method is precise and reproducible. Ibuprofen correlation coefficient is far from 1, showing a weak positive relationship. This is mainly because ibuprofen is the only compound that does not have N atoms which are more electronegative compared to O hence there is minimal attraction of the H^+ ions. Figure 5.3 did not show clear results for lower concentrations, hence Figure 5.4, a zoomed graph showing that the results are too far apart. In this study Figure 5.5 was selected for data as this shows better results at higher concentrations for Ibuprofen. This problem arose from the fact that Ibuprofen has 2 enantiomers and needed a chiral ESI enhancing chemical.

5.3.2.2 Gas Chromatography-Mass Spectroscopy (GC-MS)

All samples were derivatised using BSTFA reagent which results in a silylation chemical reaction. This process involves substitution of the active hydrogen (-OH, -COOH, -NH and -SH groups) by a silyl group to make it less polar, more volatile and stable for GCMS analysis (Orata, www.intechopen.com; Chen et al*.,* 2007). Schummer et al., 2007 reported that BSTFA was mostly used in the analysis of pharmaceuticals, anti-inflammatory drugs and other contaminants. However not all drugs in this study showed positive results after derivatisation.



Equation: Silylation reaction using N, N-bis(tri methyl-silyl)trifluoro-acetamide: TMS = Si(CH3)3, Y = O, S, NH, NR`, COO, R, R` = Alk, Ar. Khunel et al., 2007.

Caffeine and acetaminophen were not easily detectable. The caffeine peak was skewed and not sufficient to give the total peak surface area. This was as a result of the unavailability of active hydrogen for silylation. Caffeine has no hydrogen attached; hence this process was not effective for its analysis. The hydrogen in acetaminophen is not accessible as there is delocalisation by the benzene ring as well as electron withdrawing groups, i.e. -NH and the O making the hydrogen for silylation unavailable. The other two compounds were easily derivatised and the peaks for the daughter and parent ions were symmetrical.

Below are representative chromatograms with each retention time window and the calibration curve of the standard mixture of the pharmaceutical compounds which were analysed using GC-MS operated in SRM mode.

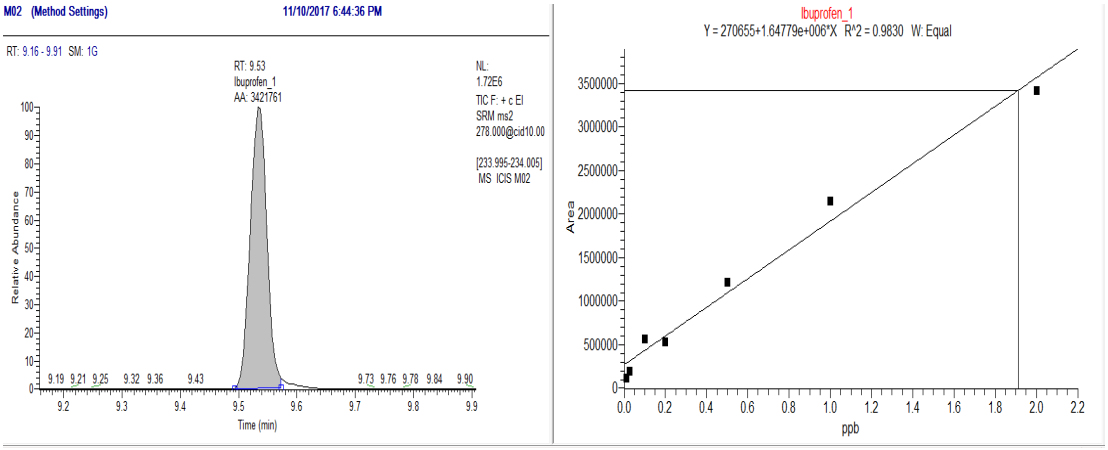


Figure 5.8: Ibuprofen 1 relative abundance against time (A) and Ibuprofen 1 peak area against concentration (B)

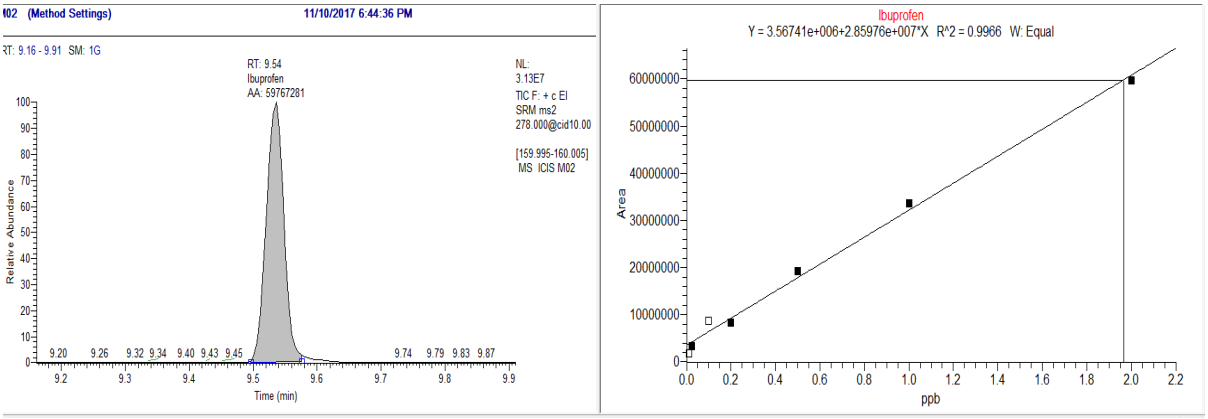


Figure 5.9: Ibuprofen relative abundance against time (C) and Ibuprofen peak area against concentration (D)

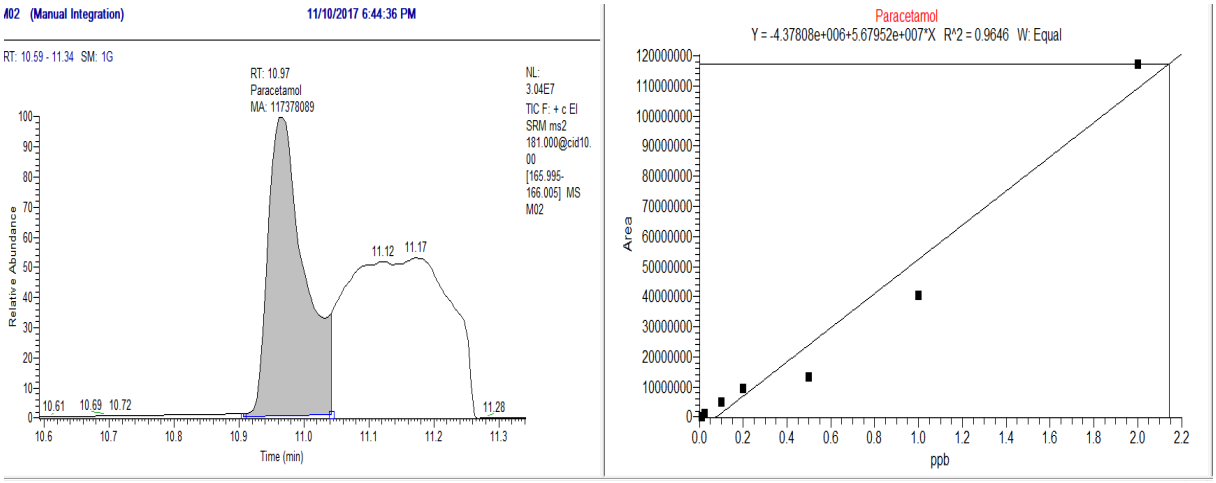


Figure 5.10: Acetaminophen Relative abundance against time (E) and Acetaminophen I Peak area against concentration (F)

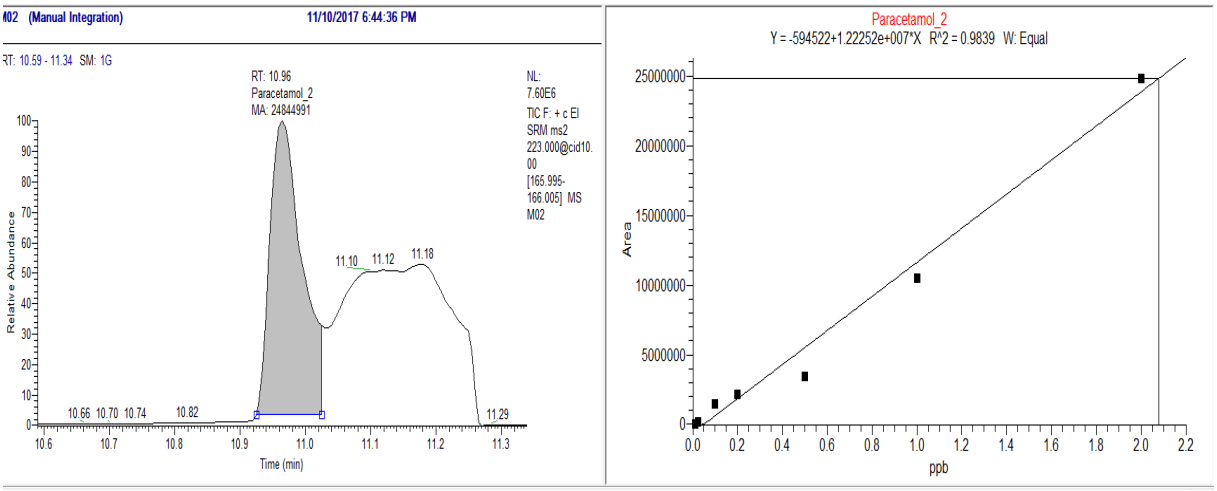


Figure 5.11: Acetaminophen 2 Relative abundance against time (G) and Acetaminophen 2 Peak area against concentration (H)

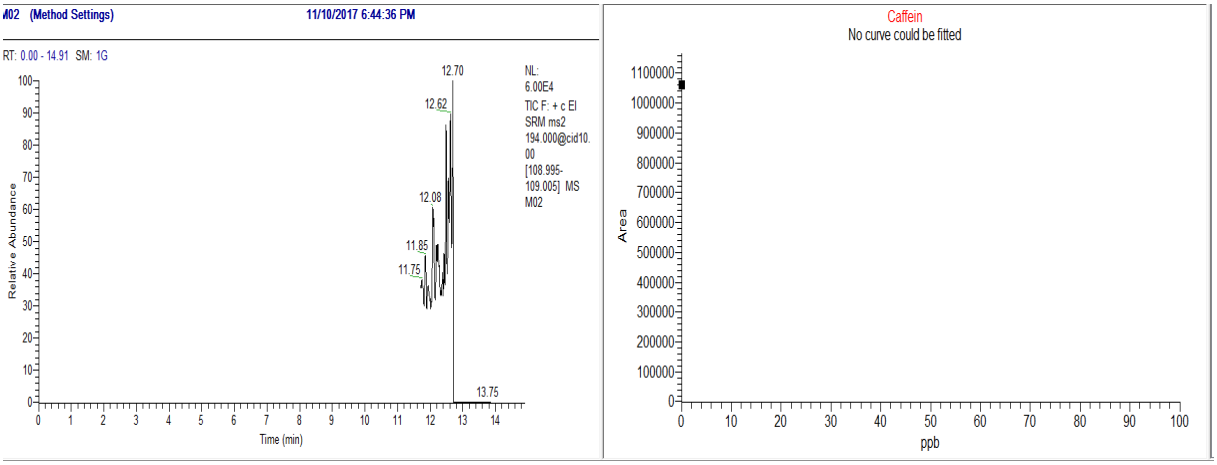


Figure 5.12: Caffeine Relative abundance against Time (I) and Caffeine Peak area against concentration (J)

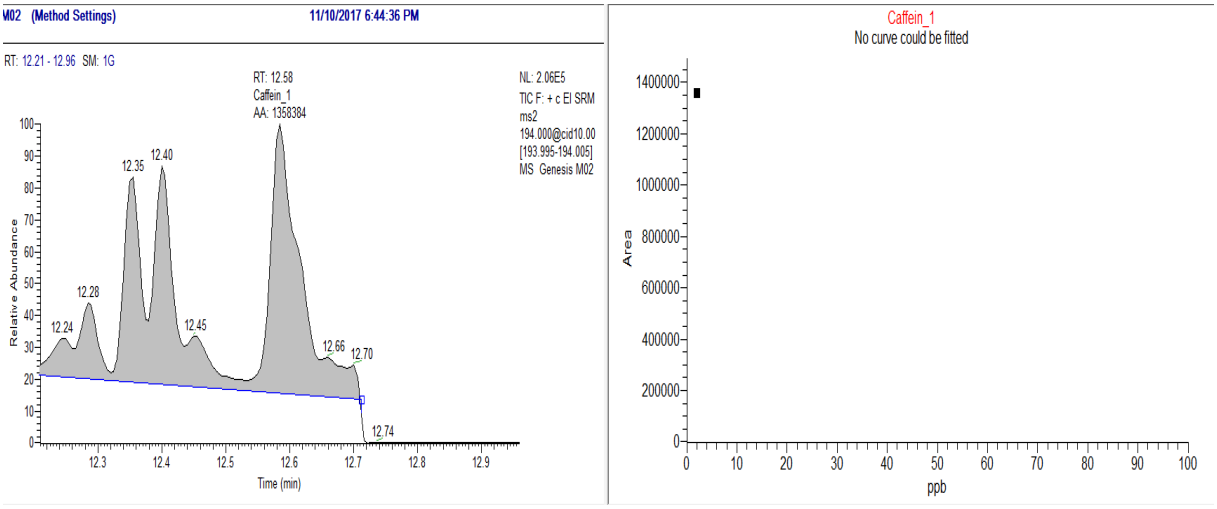


Figure 5.13: Caffeine 1 Relative abundance against time and Caffeine 1 (K) Peak area against concentration (L)

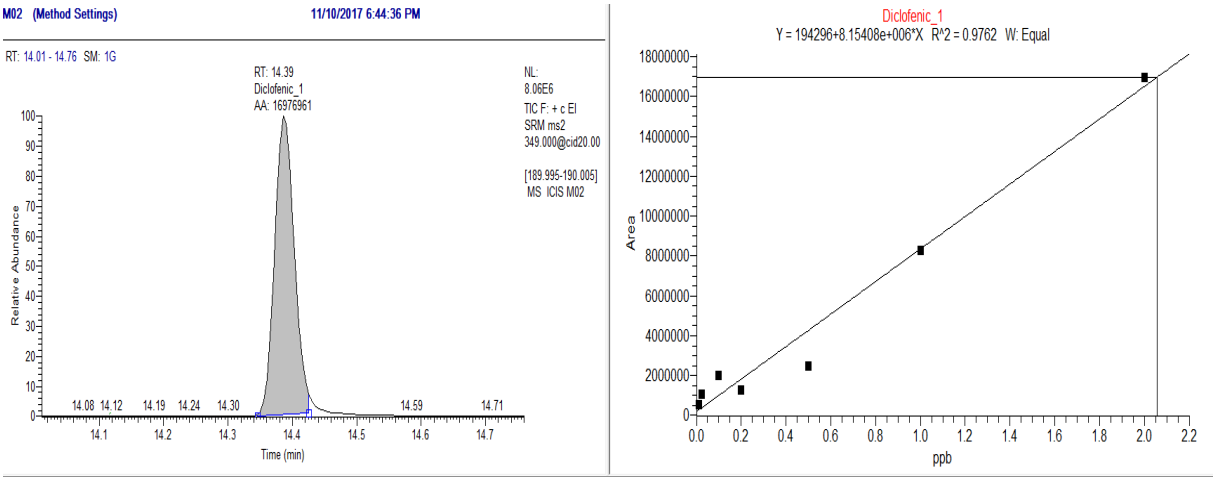


Figure 5.14: Diclofenac 1 Relative abundance against Time (M) and Diclofenac 1 Peak area against Concentration (N)

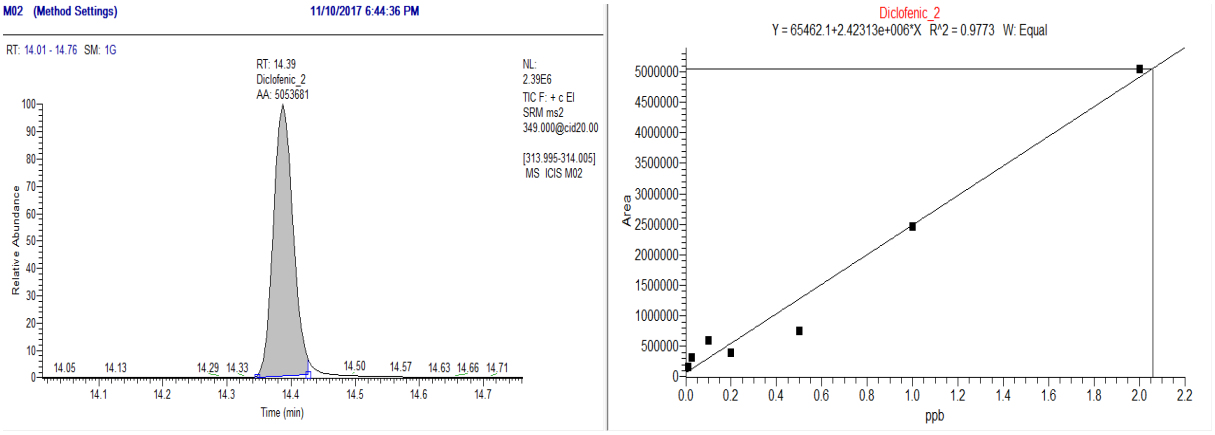


Figure 5.15: Diclofenac 2 Relative abundance against Time (O) and Diclofenac Peak area against concentration (P)

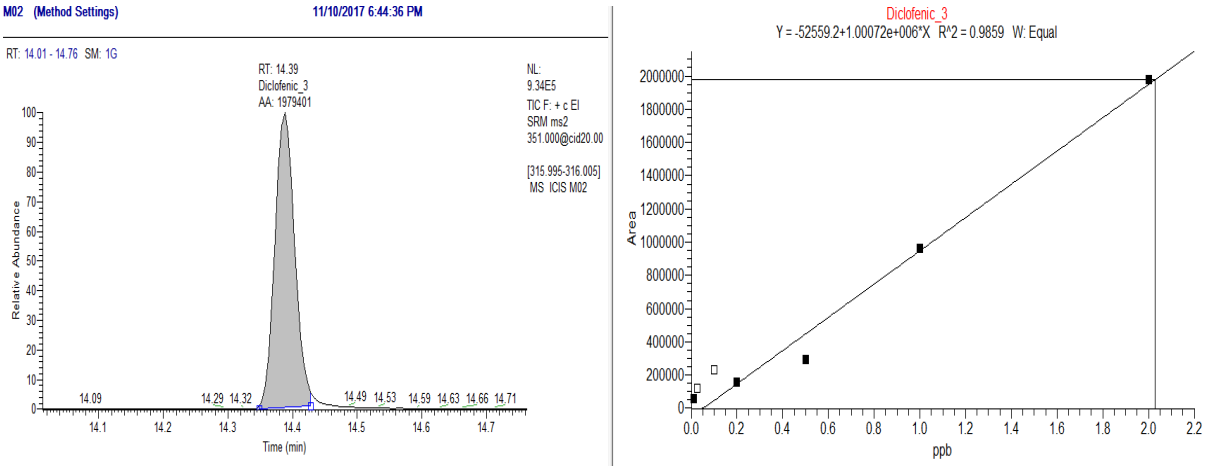


Figure 5.16: Diclofenac 2 Relative abundance against Time (Q) and Diclofenac 3 Peak area against concentration (R)

Table 5.4: Retention times, linearity (regression coefficient), detection and quantification limits of detection (LOD, LOQ) of the GC-MS method

Compound	Retention time	Regression line	LOD	LOQ
Ibuprofen	9.54	0.9966	3.60198E-15	1.09151E-14
Ibuprofen 1	9.53	0.9830	0.447279716	1.355393078
Acetaminophen	10.97	0.9646	-	-
Acetaminophen 2	10.96	0.9839	-	-
Caffeine	12.7	-	-	-
Caffeine 1	12.58	-	-	-
Diclofenac 1	14.39	0.9762	2.84828E-15	8.63115E-15
Diclofenac 2	14.39	0.9773	8.54926E-15	2.59069E-14
Diclofenac 3	14.39	0.9859	4.78E-15	1.45E-14

5.3.2.3 Retention times

The results show that ibuprofen has a low affinity for the stationary phase as it is more polar compared to the rest of the compounds. Elution followed with acetaminophen due to its failure to be derivatised and hence it had low affinity to the stationary phase. Caffeine is non-polar and had some affinity for the stationary phase though derivatisation failed. Diclofenac was eluted last because of its high molecular weight and the modification process through derivatisation process increased its affinity towards the stationary phase. The order of elution was ibuprofen> acetaminophen > caffeine> diclofenac. The standard deviation of the obtained was 0.00056 showing that the analyte concentration is close to the mean, and hence GC-MS is sensitive and repeatable. The relative standard deviation criteria for acceptance is >2% and in this study the value was 0.72% and hence showing that the method is precise and reproducible. Figure 5.12 does not show a distinct peak and as a result does not have a graph. This factor was due to the fact that no internal standard was used in the experiment and hence the instrument had difficulty measuring the Caffeine.

5.3.2.4 Surface Enhanced Raman Spectroscopy

Raman spectrum comprises wavelength distribution of peaks equivalent in character to molecular vibrations specific to the analyte being characterised (Ferraro, 2003). Surface-enhanced Raman spectroscopy (SERS) is an advanced Raman spectroscopy technique used to detect acetaminophen, using silver nanoparticles as SERS active materials to enhance the Raman signal. Figure 5.17 shows the difference in Raman signal between unmodified PET and PET modified with silver nano particles.

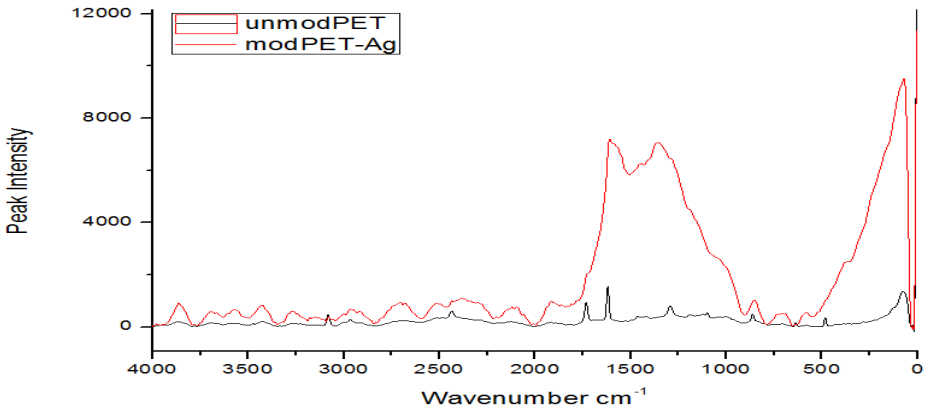


Figure 5.17: UnmodPET and modPET-Ag surfaces

The above graph shows that the Raman peak intensity for the modPET-Ag was higher than the unmodPET surface due to the Ag nanoparticles on the modPET which then was able to enhance the Raman signal. This validates use of the PET membrane as the membrane's Raman scattering will not interfere with the analyte Raman scattering. Figure 5.18 shows the spectra obtained for two concentrations of a mixture of the compounds ibuprofen, caffeine, diclofenac and paracetamol/acetominophen which were analysed on the SERS platform by Raman spectroscopy.

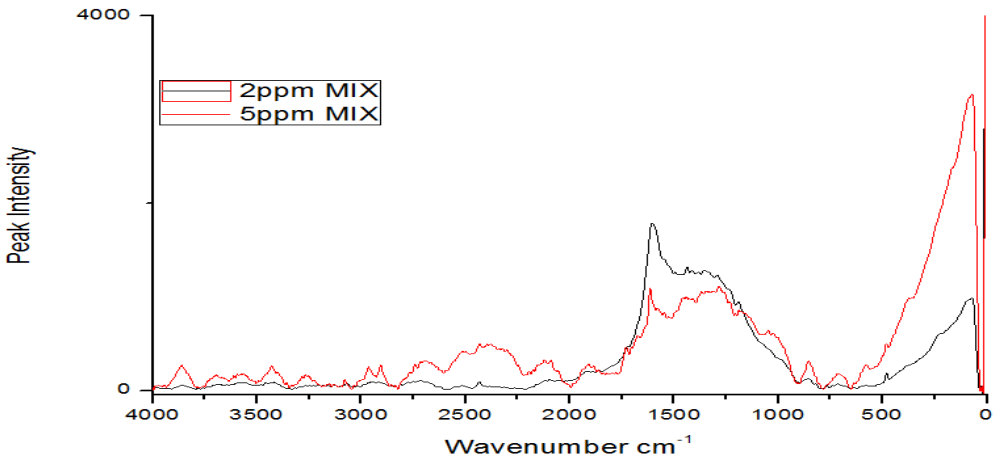


Figure 5.18: Peak intensity against Wavenumber for mixtures (MIX) 2 ppm and 5 ppm

The above graphs show that for mixtures (MIX) of standard samples at 2 ppm and 5 ppm, SERS was not able to distinguish distinct peaks for each individual pharmaceutical drug but did give a stronger signal in certain regions of the spectra, for the higher 5 ppm concentration of the mixture. There was an overlap of bonds for all the drugs under study illustrated in Table 5.5: hence identifying the analytes in a mixture was impossible, without prior separation. This shows that the SERS platform by itself is not able to resolve a mixture of compounds. It however could give a rapid fingerprint of the mixture showing that there were low levels of contaminant analytes present. But identification and quantification requires the ability to discriminate between compounds. Although low concentrations of analytes could be visualised easily via the SERS platform, the developed SERS platform can only achieve identification and quantification for single components thus must

be coupled to a chromatographic separation tool for separation of mixtures, just as in the case of MS detection systems that are always coupled to GC or HPLC for prior analyte separation.

Table 5.5: Bonds in Ibuprofen, Caffeine, Diclofenac and Paracetamol

Compound	Bonds
Ibuprofen	C-C, C=C, C-H, C=O, O-H
Caffeine	C-N, C-O, C=O, C=C, C-C
Diclofenac	C-C, C=C, N-H, C-N, C-H, C=O, C-O, C-Cl
Paracetamol	C-C, C=C, C-N, C=O, O-H

In the LC-MS analysis it was observed that ibuprofen could only be analysed at higher concentration and hence ibuprofen was analysed using the SERS platform to see if its detection would face the same challenge. The SERS peak intensity of Ibuprofen against Raman shift at different concentrations between 0.1-5 ppm is shown in Figure 5.19.

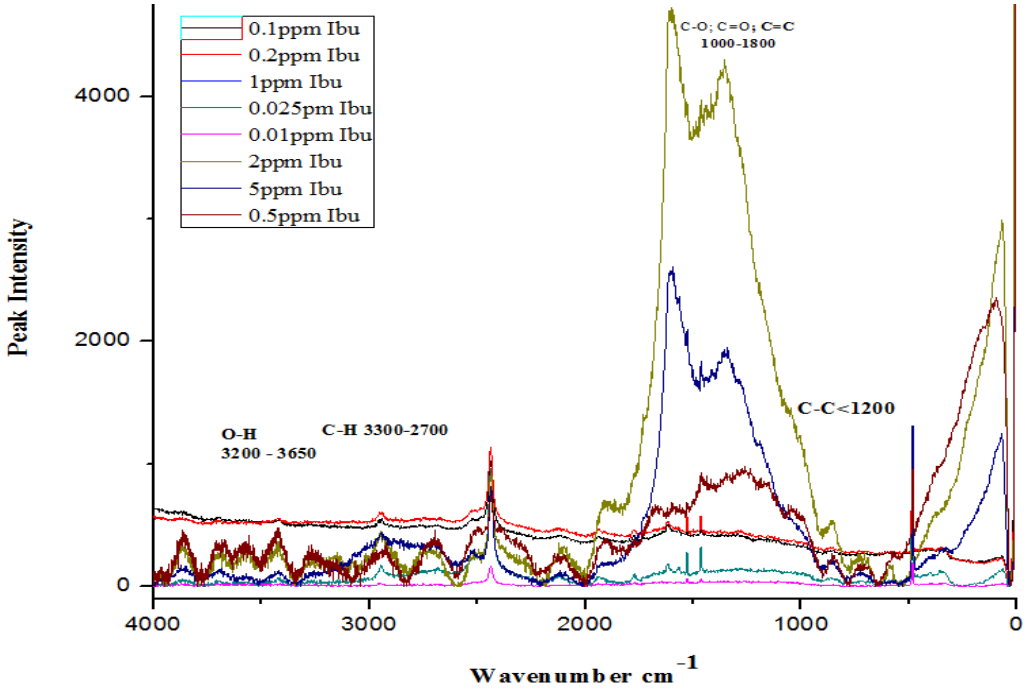


Figure 5.19: Peak intensity of Ibuprofen against Raman shift at different concentrations

The above graph (Figure 5.19) shows that there is an increase in the peak intensity due to an increase in Raman signal for every increase in concentration of ibuprofen. The Raman peaks were distinct and distinguishable for each bond in ibuprofen samples at different concentrations. The above graph (Figure 5.18) though was showing 7 graphs instead of 8. The reason was that the graph at 5 ppm was not measurable as the Raman signal was more sensitive to low concentrations and hence gave poor detection at high concentrations of ibuprofen, in contrast to the HPLC system that was only accurate for concentrations above 5 ppm.

Table 5.6: The functional groups in Ibuprofen with the expected theoretical wavenumbers

Ibuprofen functional bonds	Theoretical Wavenumber cm^{-1}
C = C	1 660
C = O	1 400-1 700
C - C	1 200
C - O	1 100
C - H	2 800-3 000
O - H	3 400-3 600

The above table (Table 5.6) shows the theoretical peaks of ibuprofen expected from the graph. The C = C and the C = O are in the same region and hence the observed peaks. Similarly the C - C and the C - O bonds are in the same region and as a result the peak is detected in the same region. The C - H bond ranges from 2 800-3 000 and appears as a broader peak. This is because there are 2 sources of the C -H bonds, that is from methanol and ibuprofen. The O - H bonds shows a broader, stronger peak as there are three sources of the O - H bond, from ibuprofen, methanol and H_2O. It is not possible to obtain definite frequencies/position for a particular bond for Raman since there is vibration of all bonds when the analyte bonds are excited. A distinct wavenumber showing all the peaks was obtained and used to plot a calibration curve at the C = O frequency (1 400-1700 cm^{-1}) which is characteristic for the ibuprofen (Figure 5.20)..

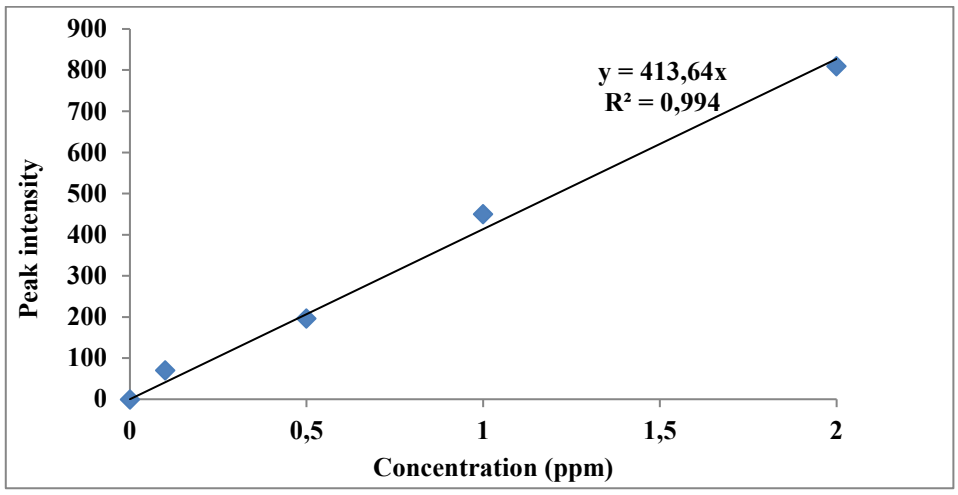


Figure 5.20: Calibration curve, Peak intensity vs Concentration of Ibuprofen

The calibration curve was obtained from the peak with a frequency at 1400-1700 cm^{-1} as it was clear and distinct showing a steady increase concomitant with the increase in concentration.

Table 5.7: Retention times, linearity (regression coefficient), detection and quantification limits of detection (LOD, LOQ) of the SERS method

Compound	Regression line	LOD(ppm)	LOQ(ppm)
Ibuprofen	0.994	0.298945237	0.905894657

The relative standard deviation was 3.5% showing a degree of inaccuracy and hence the SERS method is not highly repeatable and reproducible for measuring low concentrations accurately. The high value of 3.5% is not within the acceptable range of >2% and the variable result is ascribed to the uneven layer of the Ag nanoparticles in these SERS platforms which had not been Plasma treated and hence gave inaccuracies in Raman signal.

5.3.2.5 Surface Enhanced Raman Spectroscopy using Plasma-treated track-etched PET membrane

The peaks of the Raman spectrum in Table 5.8 were used to ascertain the presence of acetaminophen on the prepared surfaces of unmodified track-etched PET membrane (ConPET), Plasma-treated track-etched PET membrane (PlasmaPET) and Plasma-treated silver-coated track-etched PET membrane (PlasAgPET).

Table 5.8: Peaks in Raman spectrum of acetaminophen

Proposed bond	**Spectra peak cm^{-1}**
C-O	854, 861, 1170
C-N	1245, 1281, 1328
C-C	1557, 1565, 1576
C=C	1608, 1615, 1623
C=O	1646, 1650

The detection of the molecules of acetaminophen on the plasma treated silver-coated track-etched polyethene terephthalate membrane was carried out at ambient conditions. A solution of 20 µL of SPC aqueous sample was dropped and dried on the surfaces of the selected samples. The Raman spectra of acetaminophen detected on the surface of silver-coated track-etched polyethylene terephthalate (PET) membrane (PlasAgPET), unmodified track-etched PET (ConPET) and plasma-treated track-etched polyethene terephthalate (PET) membrane (PlasmaPET) are shown in Figure 5.20.

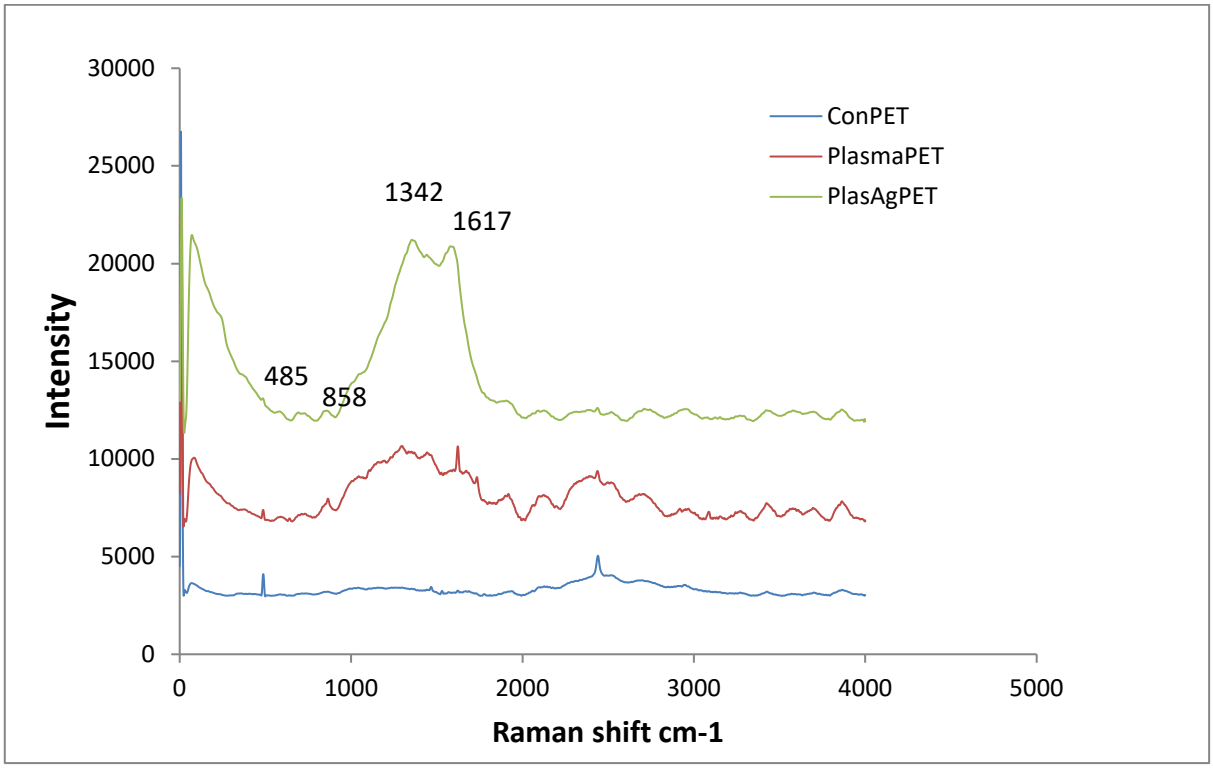


Figure 5.21: Raman spectra of SPC aqueous sample containing acetominophen on the surface of (ConPET (unmodified track-etched PET) membrane, PlasmaPET and PlasAgPET (Plasma-treated silver-coated track-etched PET membrane)

Figure 5.21 reveals that the effect of plasma treatment is actually significant, as there is an apparent modification to the plasma-treated track-etched polyethene terephthalate (PET) membrane (PlasmaPET) when compared to the control sample ConPET. The characteristic peaks of acetaminophen could not be observed among the prominent peaks of the polyethene terephthalate membrane before plasma treatment, thus, in the case of the control sample, ConPET, which is the unmodified track-etched PET membrane, no characteristic peaks of acetaminophen could be observed. This could be attributed to lack of SERS signals on the surface of sample ConPET membrane for acetaminophen. Therefore it is concluded that the lack of silver nanoparticles is the main cause of the lack of acetaminophen peaks for the sample ConPET. In the case of the plasma treated sample prior to the immobilisation of Ag, the acetominophen peaks at 1342 and 1617 cm^{-1} became evident, even without silver, showing that the plasma treatment enhanced the signal even without the plasmon resonance of the silver. Furthermore, the graph also shows that the acetominophen peak intensity on the plasma-treated, DETA activated, silver-coated track-etched PET membrane is higher than both the cases of ConPET (unmodified membrane) and Plasma-treated PET membrane, due to the silver nanoparticles immobilised on the surface of the PlasAgPET membrane. This validates the use of plasma and amino anchored silver nanoparticles on the membrane as the membrane's Raman scattering will not interfere with the analyte's Raman scattering. It is important to note that when assessing the Raman signal intensity of acetaminophen on a porous sample like the PlasAgPET (a plasma-treated track-etched silver-coated PET membrane), most of the acetaminophen vibrational bands on PlasAgPET are rather weak. The limited SERS intensity and the low spectral intensities could be due to the loss of some molecules of acetaminophen that may leach through the pores or could be due to the quality of the silver film.

The detection of acetaminophen on the surface of plasma-treated silver-coated track-etched polyethene terephthalate (PET), plasma-treated PET or unmodified track-etched PET was faced with certain bottlenecks.

One of the main limitations of Raman Spectroscopy is that the material to be analysed must be Raman active. If it is not active, there will be no measurement. Other limitations are that if the sample/compound is new and there is no data, similar compounds/samples with similar characteristics and molecular structure have to be used as standard. This will only give an estimation of the results and will not be able to give the exact result thereby not being able to explain the shift in the measurements. If the Raman energy provided is too high it may damage the material, especially biological compounds, hence the parameters have to be within the range. If the energy supplied by the Raman is too low then the material energy will be very low, hence there will not be any distinct peaks and as a result inaccurate results. The Raman spectrometer used for detection had limited options, for instance it was limited to one laser option of excitation wavelength of 532 nm, and lacked software to calculate peak heights and areas.

5.3.4 Summary

GC-MS and LC-MS have been shown to be the most reliable methods for qualitative and quantitative analysis. However a comparison of the two methods in this study showed that LCMS is the best method for identifying and quantifying certain selected pharmaceutical compounds. This is evidenced by the failure of some xenobiotics, i.e. acetaminophen and caffeine to derivatise in the GC-MS analysis and hence poor signals were observed, whilst all compounds tested were analysed easily with LC-MS. A further study on the best derivatising agent could be done to improve GC-MS analysis. Of importance is the observation that ibuprofen can easily be analysed using LC-MS at higher concentrations, above 5 ppm. Regarding the analysis GC-MS requires modifying the compound to a volatile compound and as a result the process becomes tedious and slow compared to LC-MS. Cost wise GC-MS analysis is cheaper compared to LC-MS. SERS as the new method of qualitative and quantitative analysis under investigation in this study performed very well for specific single compounds and gave rapid fingerprinting capability for mixtures. The process of membrane preparation for Raman analysis was tedious but the analysis of the samples was very quick. The method of analysis does not require a lot of destructive solvents or derivatisation, merely a droplet of the analyte sample is required for analysis and hence SERS can be a better way of rapid analysis with suitable standards. SERS was designed for analysis of very low concentrations and accordingly this study found also found better analysis results for ibuprofen at much lower concentrations. A comparison of the regression line, LOD & LOQ for LC-MS, GC-MS and SERS is presented in Table 5.9.

Table 5.9: Comparison of the Regression line, LOD & LOQ for LC-MS, GC-MS and SERS

Instrument	Compound	Regression line	LOD	LOQ
SERS	Ibuprofen	0.995341319	0.298945237	0.905894657
LCMS(2017)	Ibuprofen 1	0.8245	1.05453E-05	3.19555E-05
LCMS(2019)	Ibuprofen 1	0.989834	1.45565E-06	4.41106E-06
GC-MS	Ibuprofen	0.9966	3.60198E-15	1.09151E-14
GC-MS	Ibuprofen 1	0.9830	0.447279716	1.355393078

Looking at the above table (Table 5.9) LC-MS shows a poor regression line value for Ibuprofen compared to SERS and GC-MS. This is attributed to the fact that LC-MS can only detect high concentrations of 5 ppm and above. This phenomenon is due to the two isotopes of Ibuprofen, the R and S enantiomers of Ibuprofen. Determination of Ibuprofen can be enhanced by using a chiral ESI enhancing reagent such as DAPAP (1-(4-dimethylaminophenylcarbonyl)-3-aminopyrrolidine) to improve detectability in the positive ESI MS/MS mode and also the separation of the enantiomers. The low value of the regression line of the earlier (2017) analysis is mainly due to interferences by the other pharmaceutical compounds. The regression lines for SERS and GC-MS are close to 1 showing that the methods of analysis are efficient and very sensitive. The analysis of Ibuprofen can also be improved for both LC-MS and GC-MS by including an internal standard which will enhance detection of the drug. The Table above also shows that the LOD and LOQ for GC-MS are lower than the other instruments. This is attributed to the fact that in the experiment the standard sample in GC-MS is run first then followed by the sample, whereas in LC-MS the sample is run without the need to run the standard. This shows that GC-MS is able to detect very low concentrations of samples of the Ibuprofen compared to the other methods of analysis. However the LOD and LOQ for SERS are higher than GC-MS and LC-MS showing that the method can still be improved to obtain better values.

It is important to emphasise the value of SERS as a screening tool in the determination of persistent organic pollutants; in which case the PET membrane was used to pre-concentrate organic pollutants of low concentration by retaining them on the PET modified surface. The pre-concentration ability of the membrane is dependent on the permeability of the membrane material; nevertheless, in this case, droplets of the pollutant of interest were introduced on the modified PET membrane and allowed to dry. The developed method based on pure analyte sample for SERS analysis was able to quantify and identify ibuprofen validating the procedure as being comparable to GC-MS and LC-MS in identifying and quantifying selected analytes as was illustrated by the comparable LODs and LOQs. However the SERS method is not repeatable as inconsistences arose from the uneven quality of the enhanced silver coated plasmon surface. The PET track etched membrane is very delicate and any damage to it may affect the composition of the nanoparticles on the surface and as result the signal produced. The other challenge is that there may be loss of sample through the pores in the track etched membrane and may affect the final reading of the results. The SERS platform could be improved by using a solid support such as quartz that is coated with a certain amount of nanoparticles, thereby standardising the surface coating. Lastly, SERS has major advantages over the GC-MS and LC-MS which are use of a solvent free method making it an environmentally friendly, non-destructive analysis and trace level analysis via nanomaterial technology. SERS has the ability to measure extremely low amounts of organic compounds. In the experiment done, SERS was not able to detect higher concentrations of Ibuprofen at 5 ppm

but at lower concentrations, whereas LC-MS is only able to measure Ibuprofen at 5 ppm and higher concentrations. Hence, the SERS platform was found to be efficient in detection of very low quantities of analyte sample. This method is very useful as it requires very little sample and little sample preparation. The other methods are tedious and require intensive sample preparations whereas SERS offers a simple, fast and cost effective preparation process. The Raman instrument comes in two forms, a portable machine and the desktop equipment. With the use of the portable machine, on site measurements can be taken without the need of sample transference that could result in loss of sample. In conclusion SERS is the most cost effective, efficient, rapid and reliable screening tool in the determination of persistent organic contaminants.

5.4 SUMMARY

The fabricated silver-coated track-etched PET membrane was used successfully as a platform to detect acetaminophen. However the SERS method was not highly reproducible as inconsistences arose from the preparation of the enhanced Ag coated surface. The quality of the Ag layer on the SERS membrane needs further optimisation, so as to remove inconsistencies in the layer thickness which impacted upon the replicability of the Raman signal. SERS has major advantages over LC-MS, which are the use of a solvent free method making it environmentally friendly, non-destructive analysis protocol, and rapid screening or trace level analysis capability via nanomaterial technology. The developed method based on pure analyte samples for SERS analysis showed that the platform developed was able to identify and quantify ibuprofen. The study also showed for the first time that not all ibuprofen peaks in the Raman signal could be used for a linear correlation with analyte concentration. Validating the procedure as comparable to GC-MS and LC-MS for identifying and quantifying various selected compounds such as acetominophen, diclofenac and caffeine was illustrated by the comparable LODs and LOQs. GC-MS methods of derivitisation proved to be ineffective for some of the selected analytes. The SERS method should be used in conjunction with a chromatographic separation method in order to elute and separate multiple components in a mixture prior to their identification and quantification using a standard calibration curve. In future, further efforts will be placed upon the necessary separation of multiple components.

6 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSIONS

The study outcomes have shown that the surface of a track-etched polyethene terephthalate (PET) membrane can be modified with diethylenetriamine (DETA) via a wet chemistry technique, aminolysis. The modified track-etched PET membrane surface was characterised by the ester bond scission and formation of an amide bond (C-N). This was confirmed by Fourier transform infrared (FTIR) and X-ray photoelectron spectroscopy (XPS) spectra. The surface of PET membrane was modified successfully with DETA. The surface modification brought about changes in terms of wettability of the surface as evidenced by the contact angle measurements outcome. The chemistry of the surface was also changed by the introduction of amide bonds and changes in chemical states of carbon (C1s) following ester bond scission and formation of amide bond which resulted in a percentage increase of C 1s in C-C chemical state and a decrease in the percentage in O-C=O chemical state as shown in XPS data. The observance of a transmittance in FTIR spectrum of amine-modified track-etched PET membrane at 1578 cm^{-1} is complementary to XPS data. Further to wet chemistry modification of the surface of the PET membrane, the surface was coated successfully with immobilised silver nanoparticles via bonding with the nitrogen of DETA. The silver nanoparticles were successfully synthesised by reducing silver nitrate with trisodium citrate that served as a reducing and stabilising agent. The colloidal silver nanoparticles were stable following their zeta (ζ) potential values of more than minus 20 mV. The transmission electron microscopy images of colloidal silver nanoparticles showed that they were spherical in shape with an average size of 46 nm. The plasmonic peaks of silver solution and silver-coated track-etched PET membranes were observed between 400 and 420 nm which is typical of silver nanoparticles synthesised via reduction by trisodium citrate. The scanning electron microscopy images of the silver-coated track-etched PET membranes showed successful immobilisation of silver nanoparticles on the surface. The scanning electron microscopy (SEM) images showed silver nanoparticles sizes ranging between 28 and 68 nm. The SEM results were also in agreement with transmission electron microscopy (TEM) results regarding the spread in size of the silver nanoparticles as measured by ImageJ software. The SEM results also showed a trend in the increase of silver nanoparticles size with an increase in the time of immobilisation. Furthermore, the XPS results complemented the SEM results and UV-vis results regarding the number of silver nanoparticles immobilised on the surface where XPS data showed an increase in the percentage from 2.53 to 5.20% of silver (Ag3d5/2) relative to the increase in time of immobilisation from 10 minutes to 30 minutes. The thermal property of the PET membrane was found not to have been affected by the wet chemistry of aminolysis and immobilisation of silver nanoparticles at 90°C. The thermogravimetric analysis (TGA) showed that the amine-modified silver-coated track-etched PET membrane weighed 6% more than the amine-modified track-etched PET membrane without silver and 10% more than unmodified track-etched PET membrane. The extra weight on silver-coated track-etched PET membrane was due to silver nanoparticles that were immobilised on the surface. The Raman spectra of the silver-coated track-etched PET membrane confirmed immobilisation of a layer of silver nanoparticles that suppressed the Raman signal of polyethylene terephthalate. The more silver nanoparticles that were immobilised on the surface of PET membrane, the more the reduction of interference was observed

by suppression of the PET scattering signal. This was found to be of importance as the analyte on the surface could be detected by Raman without interference from the PET membrane Raman spectrum.

When analysing acetaminophen using a submicron sized pore track-etched PET membrane that was amine modified with 75% DETA for 24 hours and immobilised with silver nanoparticles for a duration of 30 minutes, the silver-coated track-etched PET membrane was successfully used to detect acetaminophen as low as 0.151 part per million (ppm) which was superior to the detection limit of HPLC-MS. In comparison to a quartz platform which was non-porous, it was observed that the spectra of acetaminophen detected from the surface of silver-coated track-etched PET membrane were of lower intensity. This is attributed to the loss of some of the acetaminophen molecules that leached through the pores of the track-etched PET membrane and indicate that smaller track membrane pores would be of benefit to prevent loss of analyte during the preconcentration step. The lower peak intensity could also have been from inconsistent Ag film quality. The unmodified track-etched PET membrane did not show any peaks of acetaminophen in the Raman spectrum obtained, which was mainly of PET membrane itself. It was observed that as the concentration was reduced from 15.10 ppm to 0.151 ppm of acetaminophen the Raman scattering intensity was also reduced indicating quantitative correlations. Such data leads to the conclusion that silver nanoparticles on the silver-coated track-etched PET membrane enhanced the weak Raman signal of acetaminophen to the level that was observed and analysed. It is further concluded that the Raman spectra intensity depends linearly on the concentration of the analyte for selected peaks and could offer a rapid route to calibrate and quantify the analyte if the area under the prominent peaks could be calculated as is done on gas chromatography. Also to be considered in quantification using Raman spectra intensity is the enhancement factor generated by the SERS active materials. Furthermore, literature claims that it is cumbersome to quantify using Raman spectroscopy since it requires special calculations of areas under the spectral peaks, which sometimes overlap and could result in confusions and errors. Although the fabricated silver-coated track-etched PET membrane was used successfully as a platform to rapidly detect acetaminophen, the detection capability does not yet surpass those of established analytical techniques such as gas chromatography coupled with mass spectrometry (GC/MS).

With reference to the literature review, Raman spectroscopy using the drop and dry method could be less sensitive than gas chromatography coupled with mass spectrometry (GC/MS), as the latter is capable of detecting pollutants up to 90 ng. But the latter method requires pre-concentration and derivatisation of the analyte, followed by chromatographic separation, and identification of components by mass spectrometry, where after dilution factors are integrated into the data workup, whereas in this study using Ag-PET and SERS no pre-concentration or derivatisation of the analyte was needed, showing the benefit of this approach.

In calibrating the acetaminophen signal, it was observed that as the analyte concentration was increased from 0.151 ppm to 15.10 ppm the Raman scattering intensity also linearly increased for some peaks. The correlation of the Raman peak intensity heights and concentration of acetaminophen for C-O bond vibrations at Raman shift of 861 cm^{-1} had a linear response. The other bond vibrations C-N at 1328 cm^{-1}, C=C at 1608 cm^{-1} and C-O at 1170 cm^{-1} showed a non-linear response. The best correlation from the selected peak when extrapolated gave 0.0755 mg/L as the limit of detection for acetaminophen using a track-etched PET membrane that was amine modified with 75% DETA for 24 hours and immobilised with silver nanoparticles for

a duration of 30 minutes. Such data leads to the conclusion that silver nanoparticles on the silver-coated track-etched PET membrane suppressed the PET peaks and enhanced the analyte's Raman signal to the level that was observed. It is further concluded that the Raman spectra intensity depended linearly on the concentration of the analyte and could offer a rapid route to calibrate and quantify the analyte if the area under the selected peaks could be calculated as is done on gas chromatography, or by use of a calibration curve based upon selected peaks as was demonstrated in this study. This finding has not been previously reported in literature and therefore is a new observation, made by this study. The challenge with the peak intensity heights method for calibration is that the proportionality does not seem to be the same for all peaks.

The plasma treatment of the tracketched membranes was useful to enhance the Raman signal. Acetominophen peak intensity on the plasma-treated, DETA activated, silver-coated track-etched PET membrane was higher than both the cases of ConPET (unmodified membrane) and Plasma-treated PET membrane, due to the silver nanoparticles immobilised on the surface of the PlasAgPET membrane. The unmodified track-etched PET membrane (ConPET) did not show any peaks of acetaminophen in the Raman spectrum obtained. This validates the use of plasma and amino anchored silver nanoparticles on the PET membrane as the membrane's Raman scattering did not interfere with the analyte's Raman scattering. However, the modified track-etched membrane, which was used to detect acetaminophen, was not yet fully optimised since the peaks of the Raman spectra of acetaminophen on the surface of plasma-treated silver-coated track-etched PET membrane (PlasAgPET) were of low intensity. This could be attributed to lack of consistency of the silver nanoparticle coating or due to the porous nature of the PlasAgPET membrane that caused the loss of certain amount of acetaminophen through the pores of the membrane.

In conclusion, effluents that are contaminated with a diversity of pollutants are notoriously difficult to monitor, since each contaminant needs to be separated from the others by a chromatographic technique, and then identified and quantified. The LC-MS results showed that it is possible to readily detect and quantify acetaminophen in a highly contaminated raw water effluent by spiking a set concentration and by standards addition. Thus the LC-MS technique remains a potent method that can be used, but with prior extraction and pre-concentration of a contaminated raw water sample, if suitable standards are available. However, in both GC-MS and LC-MS, the extraction process is cumbersome, thus avoiding this step would enable rapid analysis. The Surface-Enhanced Raman Spectroscopy platform would not require prior extraction and pre-concentration of samples and can be considered as alternative, rapid screening protocol to detect very low levels of pharmaceuticals in waste or contaminated water (See Appendix A for cost comparison).

6.2 RECOMMENDATIONS

Although the aim of the study was achieved, challenges were encountered during the SERS analysis. These include concentrating the droplet of analyte upon the surface. It was observed that the analyte droplet would slide from the surface as the Ag coated surface had a high contact angle which prevented pre-concentration to be fully achieved. The porous SERS platform should be used on a vacuum manifold to achieve the rapid pre-concentration of analytes and the hydrophilicity of the surface should be further tailored. PET polymer track etched membrane surfaces are very delicate and hence require gentle handling throughout the modification step. This meant that any defect in the membrane could affect the amount of Ag nanoparticles

immobilised upon the surface and hence affect the amount of Raman scattering produced. This can be overcome by using solid supports such as quartz decorated with nanoparticles as shown in this study, indicating that the support did not need to be based on the porous track etched membrane if pre-concentration was not needed. Inconsistencies may also arise from the amount of Ag nanoparticles deposited on the surface during preparation of the modPET-Ag surface which could vary during processing. The SERS method is significantly cheaper compared to all the methods (See Appendix A), and with the use of a solid quartz support the analysis cost can be further reduced. Use of prefabricated solid supports also tends to shorten the analysis time hence there will be fewer delays compared to LC-MS and GC-MS. Since the other analytical techniques are hyphenated to chromatographic techniques, separation of analyte mixtures vs quantification and identification was possible, whereas the SERS platform presented in this study is not hyphenated. For ease of analysis of real samples, coupling the SERS platform to suitable chromatographic techniques can aid in providing pure samples for analysis. Also for ease of calibration and calculation of LOD, LOQ in the SERS analysis a software tool such as Origin can be added to the Raman system thereby providing reliable results. Furthermore, it is advisable to explore the possibility of using chemometrics for the analysis of spectra integration and deconvolution of peaks. There were challenges in the analysis of spectra-integration and deconvolution of peaks hence Chemometrics can be used to analyse data as it can assist in giving a comprehensive and efficient way of analysing the statistical data using memory/recognition.

REFERENCES

Abdesselam, M., Ouichaoui, S., Azzouz, M., Chami, A.C. & Siad, M. (2008). Stopping of 0.3-1.2 MeV/u protons and alpha particles in Si. Nuclear Instruments and Methods in Physics Research B, 266, 3899-3905.

Abdesselam, M., Stoquert, J. P., Djebara, M., Cerruti, C., Chami, A.C. & Montgomery, P. (2011). Stoichiometry evolution of polyethylene terephthalate under 3.7 MeV He+ irradiation. Nuclear Instruments and Methods in Physics Research B, 269, 140-144.

Abdesselam, M., Stoquert, J.P., Chami, S., Djebara, M., Chami, A.C. & Siad, M. (2009). Method to measure composition modifications in polyethylene terephthalate during ion beam irradiation. Nuclear Instruments and Methods in Physics Research B, 267, 108-112.

Abou-El-Nour, K.M., Eftaiha, A., Al-Warthan, A. & Ammar, R.A. (2010). Synthesis and applications of silver nanoparticles. Arabian Journal of Chemistry, 3, 135-140.

Acharya, N.K., Kulshrestha, V., Awasthi, K., Kumar, R., Jain, A.K., Singh, M., Avasthi, D.K. & Vijay, Y.K. (2006). Gas permeation study of Ti-coated, track-etched polymeric membranes. Vacuum, 81, 389-393.

Agunbiade, F.O. & Moodley, B. (2016). Occurrence and distribution pattern of acidic pharmaceuticals in surface water, wastewater, and sediment of the Msunduzi River, KwaZulu-Natal, South Africa. Environmental Toxicology and Chemistry, 35, 36-46.

Andrade, G.F., Fan, M. & Brolo, A.G. (2010). Multilayer silver nanoparticles-modified optical fiber tip for high performance SERS remote sensing. Biosensors and Bioelectronics, 25(10), 2270-2275.

Anumol, T., Merel, S., Clarke, B. & Snyder, S. (2013). Ultra high performance liquid chromatography tandem mass spectrometry for rapid analysis of trace organic contaminants in water. Chemistry Central Journal, 7, 104-110.

Apel, P. Y., Akimenko, A. P., Blonskaya, I. V., Cornelius, T., Neumann, R., Schwartz, K., Spohr, R. & Trautmann, C. (2006). Etching of nanopores in polycarbonate irradiated with swift heavy ions at 15K. Nuclear Instruments and Methods in Physics Research B, 245, 284-287.

Apel, P.Y., Blonskaya, I., Dmitriev, S., Orelovich, O. & Sartowska, B. (2015). Ion track symmetric and asymmetric nanopores in polyethylene terephthalate foils for versatile applications. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 365, 409-413.

Apel, P., Schulz, A., Spohr, R., Trautmann, C. & Vutsadakis, V. (1997). Tracks of very heavy ions in polymers. Nuclear Instruments and Methods in Physics Research B, 131, 55-63.

Awasthi, K., Kulshrestha, V., Avasthi, D.K. & Vijay, Y.K. (2010). Optical, chemical and structural modification of oxygen irradiated PET. Radiation Measurements, 45, 850-855.

Aziz, K.H., Miessnera H., Mueller, S., Kalassa, D., Moeller, D., Khorshid, I. & Rashid, M.A. (2017). Degradation of pharmaceutical diclofenac and ibuprofen in aqueous solution, a direct comparison of ozonation, photocatalysis, and non-thermal plasma. Chemical Engineering Journal, 313, 1033-1041.

Banholzer, M.J., Millstone, J.E., Qin, L. & Mirkin, C.A. (2008). Rationally designed nanostructures for surface-enhanced Raman spectroscopy. Chemical Society Reviews, 37(5), 885-897.

Bantz, K.C. & Haynes, C.L. (2009). Surface-enhanced Raman scattering detection and discrimination of polychlorinated biphenyls. Vibrational Spectroscopy, 50(1), 29-35.

Bastus, N.G., Merkoci, F., Piella, J. & Puntes, V. (2014). Synthesis of highly monodisperse citrate-stabilized silver nanoparticles of up to 200 nm: Kinetic control and catalytic properties. Chemical Materials, 26, 2836-2846.

Bell, S.E. & Sirimuthu, N.M. (2008). Quantitative surface-enhanced Raman spectroscopy. Chemical Society Reviews, 37, 1012-1024.

Botti, S., Cantarini, L., Almaviva, S., Puiu, A. & Rufoloni, A. (2014). Assessment of SERS activity and enhancement factors for highly sensitive gold coated active materials probed with explosive molecules. Chemical Physics Letters, 592, 277-281.

Boujday, S., Chapelle, Marc Lamy de la, Srajer, J. & Knoll, W. (2015). Enhanced vibrational spectroscopies as tools for small molecule biosensing. Sensors, 15, 21239-21264.

Boxall, A.B.A., Fogg, L.A., Blackwell, P.A., Kay, P., Pemberton, E.J. & Croxford, A. (2004). Veterinary medicines in the environment. Reviews of Environmental Contamination and Toxicology, 180, 1-91.

Braun, G.B., Lee, S.J., Laurence, T., Fera, N., Fabris, L., Bazan, G.C. (2009). Generalized approach to SERS nanomaterials via controlled nanoparticle linking, polymer encapsulation, and small-molecule infusion. The Journal of Physical Chemistry C, 113, 13622-13629.

Bravo, J.C., Garcinuño, R.M., Fernández, P. & Durand J.S. (2007). A new molecularly imprinted polymer for the on-column solid-phase extraction of diethylstilbestrol from aqueous samples. Analytical and Bioanalytical Chemistry, 388, 1039-1045.

Braydich-Stolle, L., Hussain, S., Schlager, J.J. & Hofman, M.C. (2005). In-vitro cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicology Science, 88, 412-419.

Bueno, M.J., Ucles, S., Hernando, M.D. & Fernandez-Alba, A.R. (2011). Development of a solvent free method for simultaneous identification/quantification of drugs of abuse and their metabolites in environmental water by LC-MS/MS. Talanta, 85, 157-166.

Chang, B.V., Yuan, S.Y. & Chiou, C.C. (2011). Biodegradation of bisphenol-A in river sediment. Journal of Environmental Science Health A, 46, 931-937.

Chen, B., Wang, S. & Liu, R.H. (2007). GC-MS analysis of multiply derivatized opioids in urine. Journal of Mass Spectrometry, 1012-1023.

Chong, S., Zhang, G., Zhang, N., Liu, Y., Huang, T. & Chang, H. (2017). Diclofenac degradation in water by FeCeOx catalyzed H2O2: Influencing factors, mechanism and pathways. School Journal of Hazardous Materials, 334, 150-159.

Cialla, D., März, A., Böhme, R., Theil, F., Weber, K., Schmitt, M. (2012). Surface-enhanced Raman spectroscopy (SERS): Progress and trends. Analytical and Bioanalytical Chemistry, 403, 27-54.

Comerton, A.M., Andrews, R.C. & Bagley, D.M. (2009). Practical overview of analytical methods for endocrine-disrupting compounds, pharmaceuticals and personal care products in water and wastewater. Philosophical Transactions of the Royal Society of London a: Mathematical, Physical and Engineering Sciences, 367, 3923-3939.

Costa, J., Sant'Ana, A., Corio, P. & Temperini, M. (2006). Chemical analysis of polycyclic aromatic hydrocarbons by surface-enhanced Raman spectroscopy. Talanta, 70, 1011-1016.

Craig, A.P., Franca, A.S. & Irudayaraj, J. (2013). Surface-enhanced Raman spectroscopy applied to food safety. Annual Review of Food Science and Technology, 4, 369-380.

Cui, Y., Wei, Q., Park, H. & Lieber, C. (2001). Species Nanowire Nanosensors for Highly Sensitive and Selective Detection of Biological and Chemical. Science, 293, 1289-1292.

Culha, M., Cullum, B., Lavrik, N. & Klutse, C.K. (2012). Surface-enhanced Raman scattering as an emerging characterisation and detection technique. Journal of Nanotechnology, 2012, 1-15.

Cunha, S.C., Pena, A. & Fernandes, J.O. (2017). Mussels as bioindicators of diclofenac contamination in coastal environments. Environmental Pollution, 225, 354-360.

Dai, C., Geissen, S., Zhang, Y., Zhang, Y. & Zhou, X. (2011). Selective removal of diclofenac from contaminated water using molecularly imprinted polymer microspheres. Environmental Pollution 159, 1660-1666.

Dams, R., Huestis, M., Lambert, W. & Murphy, C. (2003). Matrix Effect in Bio-Analysis of Illicit Drugs with LC-MS/MS: Influence of Ionization Type, Sample Preparation, and Biofluid. Journal of the American Society for Mass Spectrometry, 14, 1290-1294.

Dauginet, L., Duwez, A., Legras, R. & Demoustier-Champagne, S. (2001). Surface modification of polycarbonate and poly (ethylene terephthalate) films and membranes by polyelectrolyte deposition. Langmuir, 17, 3952-3957.

Deldime, M., Dewez, J., Schneider, Y. & Marchand-Brynaert, J. (1995). Reactivity assay of surface carboxyl chain-ends of poly (ethylene terephthalate) (PET) film and track-etched microporous membranes using fluorine labelled-and/or 3 H-labelled derivatization reagents: Tandem analysis by X-ray photoelectron spectroscopy (XPS) and liquid scintillation counting (LSC). Applied Surface Science, 90, 1-14.

Ding, T., Yang , M., Zhang, J., Yang , B., Lin , K Li, J. & Gan , J. (2017). Toxicity, degradation and metabolic fate of ibuprofen on freshwater diatom *Navicula* sp. Journal of Hazardous Materials, 330, 127-134.

Dong, X., Ji, X., Wu, H., Zhao, L., Li, J. & Yang, W. (2009). Shape control of silver nanoparticles by stepwise citrate reduction. Journal of Physical Chemistry, 113, 6573-6576.

Drobota, M., Persin, Z., Zemljic, L., Mohan, T., Stana-Kleinschek, K., Doliska, A. (2013). Chemical modification and characterisation of poly (ethylene terephthalate) surfaces for collagen immobilization. Open Chemistry, 11, 1786-1798.

Fan, M., Andrade, G.F.S. & Brolo, A.G. (2011). A review on the fabrication of active materials for surface enhanced Raman spectroscopy and their applications in analytical chemistry. Analytica Chimica Acta, 693, 7-25.

Farre, M., Ferrer, I., Ginebreda, A., Figueras, M., Olivella, L., Tirapuc, L., Vilanovac, M. & Barcelo, D. (2001). Determination of drugs in surface water and wastewater samples by liquid chromatography-mass spectrometry: methods and preliminary results including toxicity studies with Vibrio fischer. Journal of Chromatography A, 938, 187-197.

Fatiyants, E.K., Berezkin, V. & Kagramanov, G. (2013). Methods for modification of track-etched membranes designed for separation of biological objects. Petroleum Chemistry, 53, 471-481.

Fatta, D., Nikolaou, A., Achilleos, A. & Meric, S. (2007). Analytical methods for tracing pharmaceutical residues in water and wastewater. Trends in Analytical Chemistry, 26, 515-533.

Fávaro, S.L., Rubira, A.F., Muniz, E.C. & Radovanovic, E. (2007). Surface modification of HDPE, PP, and PET films with KMnO4/HCl solutions. Polymer Degradation and Stability, 92, 1219-1226.

Fawell, J. & Ong, C.N. (2012). Emerging contaminants and the implications for drinking water. Water Resources Development, 28, 247-263.

Ferraro, J.R. (2003). Introductory Raman spectroscopy. New York: Academic press.

Garric, J., Vollat, B., Duis, K., Pery, A., Junker, T., Ramil, M., Fink, G., Ternes, T.A (2007). Effects of the parasiticide ivermectin on the cladoceran *Daphnia magna* and the green alga *Pseudokirchneriella subcapitata*. Chemosphere, 69, 903-910.

Gatidou, G., Thomaidis, N., Stasinakis, A. & Lekkas, T. (2007). Simultaneous determination of the endocrine disrupting compounds nonylphenol, nonylphenol ethoxylates, triclosan and bisphenol A in wastewater and sewage sludge by gas chromatography-mass spectrometry. Journal of Chromatography A, 1138, 32-41.

Gil, R.J., Cáceres, N., Dafouza, R. & Valcárcel, Y. (2018). Caffeine and paraxanthine in aquatic systems: Global exposure distributions and probabilistic risk assessment. Science of the Total Environment, 612, 1058-1071.

Goddard, J.M. & Hotchkiss, J. (2007). Polymer surface modification for the attachment of bioactive compounds. Progress in Polymer Science, 32, 698-725.

Gomez, M.J., Bueno, M., Lacorte, S., Fernandez-Alba, A.R. & Agüera, A. (2007). Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. Chemosphere, 66, 993-1002.

Gorham, J.M., MacCuspie, R.I., Klein, K.L., Fairbrother, D.H. & Holbrook, D. (2012). UV-induced photochemical transformations of citrate-capped silver nanoparticle suspensions. Journal of Nanoparticle Research, 14, 1139-1154.

Gros, M., Petrovi, M. & Barcel, D. (2006). Development of a multi-residue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. Talanta, 70, 678-690.

Guerrini, L., Pazos, E., Penas, C., Vázquez, M.E., Mascareñas, J.L. & Alvarez-Puebla, R.A. (2013). Highly Sensitive SERS Quantification of the Oncogenic Protein c‑Jun in Cellular Extracts. Journal of American Chemical Society, 135, 10314-10317.

Gühlke, M., Heiner, Z. & Kneipp, J. (2016). Surface-Enhanced Raman and Surface-Enhanced Hyper-Raman Scattering of Thiol-Functionalised Carotene. The Journal of Physical Chemistry. C, Nanomaterials and Interfaces, 120, 20702.

Guiloski, R.I., Piancini, L.D., Dagostim, A.C., Calado, S.L., Fávaro, L.F., Boschen, S.L., Cestari, M.M., Cunha, C. & Silva de Assis, H.C. (2017). Effects of environmentally relevant concentrations of the anti-inflammatory drug diclofenac in freshwater fish. Ecotoxicology and Environmental Safety, 139, 291-300.

Gulbranson, S.H., Hud, J.A. & Hansen, R.C. (2000). Argyria following the use of dietary supplements containing colloidal silver protein. Cutis, 66, 373-376.

Gumbi, B.P., Moodley, B., Birungi, G. & Ndungu, P.G. (2017). Assessment of nonsteroidal anti-inflammatory drugs by ultrasonic-assisted extraction and GC-MS in Mgeni and Msunduzi river sediments, KwaZulu-Natal, South Africa. Environmental Science and Pollution Research, 4, 20015-20028.

Halvorson, R.A. & Vikesland, P.J. (2010). Surface-Enhanced Raman Spectroscopy (SERS) for Environmental Analyses. Environmental Science and Technology, 44, 7749-7755.

Hao, C., Zhao, X. & Yang, P. (2007). GC-MS and HPLC-MS analysis of bioactive pharmaceuticals and personal-care products in environmental matrices. Trends in Analytical Chemistry, 26. 569-580.

Hering, K., Cialla, D., Ackermann, K., Dörfer, T., Möller, R., Schneidewind, H. (2008). SERS: A versatile tool in chemical and biochemical diagnostics. Analytical and Bioanalytical Chemistry, 390, 113-124.

Hoffmann, N. (2008). Photochemical reactions as key steps in organic synthesis. Chemical. Reviews, 108, 1052-1103.

Holčapek, M., Jirasko, R. & Miroslav, L. (2012). Recent developments in liquid chromatography-mass spectrometry and related techniques of Chromatography A, 1259, 3-15.

Hou, F., Deng, T. & Jiang, X. (2013). Dispersive liquid-liquid microextraction of phenolic compounds using solidified floating organic droplets, and their determination by HPLC. Microchimical Acta, 180, 341-346.

Houtman, C.J. (2010). Emerging contaminants in surface water and their relevance for the production of drinking water in Europe. Journal of Integrative Environmental Science, 7, 271-295.

Huh, Y.S., Chung, A.J. & Erickson, D. (2009). Surface enhanced Raman spectroscopy and its application to molecular and cellular analysis. Microfluidics and Nanofluidics, 6, 285-297.

Irena, G., Jolanta, B. & Karolina, Z. (2009). Chemical modification of poly (ethylene terephthalate) and immobilization of the selected enzymes on the modified film. Applied Surface Science, 255, 8293-8298.

Jernberg, J. (2013). Novel analytical methods for the identification of emerging contaminants in aquatic environments, Dissertation submitted to University of Helsinki, Finland.

Ji, X., Song, X., Li, J., Bai, Y., Yang, W. & Peng, X. (2007). Size Control of Gold Nanocrystals in Citrate Reduction: The Third Role of Citrate. Journal of the American Chemical Society, 129, 13939-13948.

Kanda, R. & Glendinning, R. (2011). Mass spectroscopy for environmental and waste water monitoring. Spectroscopy Europe Asia, 23, No 5.

Kan-Sen, C. & Chiang-Yuh, R. (2000). Synthesis of nanosized silver particles by chemical reduction method. Materials Chemistry and Physics, 64, 241-246.

Kauffman, J.F., Batykefer, L.M. & Tuschel, D.D. (2008). Raman detected differential scanning calorimetry of polymorphic transformations in acetaminophen. Journal of pharmaceutical and biomedical analysis, 48, 1310-1315.

Kaur, H., Bansiwal, A., Hippargi, G. & Pophali, G.R. (2017). Effect of hydrophobicity of pharmaceuticals and personal care products for adsorption on activated carbon: Adsorption isotherms, kinetics and mechanism. Environmental Science and Pollution Research, 25, 20473-20485.

Khan, Z., Al-Thabaiti, S.A., Obaid, A.Y. & Al-Youbi, A. (2011). Preparation and characterisation of silver nanoparticles by chemical reduction method. Colloids and Surfaces B: Biointerfaces, 82, 513-517.

Khunel, E., Laffan, D.D., Lloyd-Jones, G.C., Campo, T.M., Shepperson, I.R. & Agnew, J.L. (2007). Mechanism of Methyl Esterification of Carboxylic Acids by Trimethylsilyldiazomethane. Chemie International Edition, 46, 7075-7078.

Klavarioti, M., Mantzavinos, D. & Kassinos, D. (2009). Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes. Environment International, 35, 402-417.

Kleinman, S.L., Frontiera, R.R., Henry, A., Dieringer, J.A. & Van Duyne, R.P. (2013). Creating, characterising, and controlling chemistry with SERS hot spots. Physical Chemistry Chemical Physics, 15, 21-36.

Klutse, C., Mayer, A., Wittkamper, J. & Cullum, B. (2012). Applications of Self-Assembled Monolayers in Surface-Enhanced Raman Scattering, Journal of Nanotechnology, Hindawi Publishing Corporation, 2012, 1-10.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B. & Buxton, H.T. (2002). Pharmaceutical, hormones, and other organic wastewater contaminants in US streams, 1999-2000: a national reconnaissance. Environmental Science and Technology, 36, 1202-1211.

Korolkov, I.V., Mashentseva, A.A., Güven, O., Zdorovets, M.V. & Taltenov, A.A. (2015). Enhancing hydrophilicity and water permeability of PET track-etched membranes by advanced oxidation process. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 365, Part B, 651-655.

Kostopoulou, M. & Nikolaou, A. (2008). Analytical problems and the need for sample preparation in the determination of pharmaceuticals and their metabolites in aqueous environmental matrices. Trends in Analytical Chemistry, 27, 1023-1035.

Kubackova, J., Fabriciova, G., Miskovsky, P., Jancura, D. & Sanchez-Cortes, S. (2014). Sensitive surface-enhanced Raman spectroscopy (SERS) detection of organochlorine pesticides by alkyl dithiol-functionalised metal nanoparticles-induced plasmonic hot spots. Analytical Chemistry, 87, 663-669.

Kumar, S. & Chakarvarti, S.K. (2006). Electrodeposition of copper nanowires in ion-crafted membranes as templates. Digest Journal of Nanomaterials Biostructures, 1, 39-143.

Kumar, V., Sonkawade, R.G., Chakarvarti, S.K., Singh, P., Dhaliwal, A.S. (2012). Carbon ion beam induced modifications of optical, structural and chemical properties in PADC and PET polymers. Radiation Physics and Chemistry, 81, 652-658.

Lacey, C., McMahon, G., Bones, J., Barron, L., Morrissey, A. & Tobin, J.M. (2008). An LC-MS method for the determination of pharmaceutical compounds in wastewater treatment plant influent and effluent samples. Talanta, 75, 1089-1097.

Lamer, V.K. & Dinegar, R.H. (1950). Theory, production and mechanism of formation of monodispersed hydrosols. Journal of American Chemical Society, 72, 4847-4854.

Lamsal, R., Harroun, S.G., Brosseau, C.L. & Gagnon, G.A. (2012). Use of surface enhanced Raman spectroscopy for studying fouling on nanofiltration membrane. Separation and Purification Technology, 96, 7-11.

Länge, R., Hutchinson, T.H., Croudace, C.P., Siegmund, F., Schweinfurth, H., Hampe, P., Panter, G.H. & Sumpter, J.P. (2001). Effects of the synthetic estrogen 17 alpha-ethinylestradiol on the life-cycle of the fathead minnow (Pimephales promelas). Environmental Toxicology Chemistry, 20, 1216-1227.

Le Ru, E. & Etchegoin, P. (2008). Principles of surface-enhanced Raman spectroscopy: And related plasmonic effects. Oxford: Elsevier.

Lee, A., Elam, J.W. & Darling, S.B. (2016). Membrane materials for water purification: Design, development, and application. Environmental Science: Water Research & Technology, 2, 17-42.

Lee, P.C. & Melsel, D. (1982). Adsorption and Surface-Enhanced Raman of Dyes on Silver and Gold Sols. Journal of Physical Chemistry, 86, 3391-3395.

Li, D., Zhai, W., Li, Y. & Long, Y. (2014). Recent progress in surface enhanced Raman spectroscopy for the detection of environmental pollutants. Microchimica Acta, 181, 23-43.

Lindholm, P.C., Kruutinen, J.S., Ahkola, H.S., Herve, S.H. (2014). Analysis of trace pharmaceuticals and related compounds in municipal wastewaters by preconcentration, chromatography, derivatisation and separation. Analysis of Pharmaceuticals. Bio Resources, 9, 3688-3732.

Lindsey, M., Meyer, M. & Thurman, E. (2001). Analysis of Trace Levels of Sulfonamide and Tetracycline Antimicrobials in Groundwater and Surface Water Using Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry. Analytical Chemistry, 73, 4640-4646.

Lombardi, J.R. & Birke, R.L. (2009). A unified view of surface-enhanced Raman scattering. Accounts of Chemical Research, 42(6), 734-742.

Long, T.M., Prakash, S., Shannon, M.A. & Moore, J.S. (2006). Water-vapor plasma-based surface activation for trichlorosilane modification of PMMA. Langmuir, 22, 4104-4109.

Lucotti, A. & Zerbi, G. (2007). Fiber-optic SERS sensor with optimized geometry. Sensors and Actuators B: Chemical, 121, 356-364.

Ma, P., Liang, F., Wang, D., Yang, Q., Cao, B. & Song, D. (2015). Selective determination of o-phenylenediamine by surface-enhanced Raman spectroscopy using silver nanoparticles decorated with α-cyclodextrin. Microchimica Acta, 182, 167-174.

Marchand-Brynaert, J., Deldime, M., Dupont, I., Dewez, J. & Schneider, Y. (1995). Surface functionalisation of poly(ethylene terephthalate) film and membrane by controlled wet chemistry: Chemical characterisation of carboxylated surfaces. Journal of Colloid and Interface Science, 173, 236-244.

Matousek, P. & Parker, A.W. (2006). Bulk Raman analysis of pharmaceutical tablets. Applied spectroscopy, 60, 1353-1357.

McMahon, G. (2008). Analytical instrumentation: A guide to laboratory, portable and miniaturized instruments. Chichester: John Wiley & Sons.

McNay, G., Eustace, D., Smith, W.E., Faulds, K. & Graham, D. (2011). Surface-enhanced Raman scattering (SERS) and surface-enhanced resonance Raman scattering (SERRS): A review of applications. Applied Spectroscopy, 65, 825-837.

Megson, D., Reiner, E., Jobst, K., Dorman, F., Robson, M. & Focant, J. (2016). A review of the determination of persistent organic pollutants for environmental forensics investigations. Analytica Chimica Acta 941, 10-25.

Meheretu, G.M., Cialla, D. & Popp, J. (2014). Surface enhanced Raman spectroscopy on silver nanoparticles. International Journal of Biochemistry and Biophysics, 2, 63-67.

Meyers, R.A. (2000). Encyclopedia of analytical chemistry: Applications, theory and instrumentation. [Raman spectroscopy, thermal analysis, X-ray photoelectron spectroscopy and auger electron spectroscopy, X-ray spectrometry, general articles, indexes]. London: Wiley & Sons.

Miao, X., Koenig, B.G. & Metcalfe, C.D. (2002). Analysis of acidic drugs in the effluents of sewage treatment plants using liquid chromatography-electronspray ionisation tandem mass spectroscopy. Journal of chromatography A, 952,139-147.

Moeder, M., Schrader, S., Winkler, M. & Popp, P. (2000). Solid-phase microextraction-gas chromatography-mass spectrometry of biologically active substances in water samples. Journal of Chromatography A, 873, 95-106.

Mokhena, T., Jacobs, V. & Luyt, A. (2015). A review on electrospun bio-based polymers for water treatment. Express Polymer Letters, 9, 839-880.

Morelli, L., Zór, K., Jendresen, C.B. & Rindzevicius, T. (2017). Surface enhanced Raman scattering for quantification of p-coumaric acid produced by Escherichia coli. Analytical Chemistry, *89*, 3981-3987.

Mottaleb, M.A., Usenko, S., O'Donnell, J.G., Ramirez, A.J., Brooks, B.W. & Chambliss, C.K. (2009). Gas chromatography-mass spectrometry screening methods for select UV-filters, synthetic musks, alkylphenols, an antimicrobial agent, and an insect repellent in fish. Journal of chromatography A, 1216, 815-823.

Muehlethaler, C., Leona, M. & Lombardi, J.R. (2015). Review of surface enhanced Raman scattering applications in forensic science. Analytical Chemistry, 88, 152-169.

Mulamattathil, S.G., Bezuidenhout, C., Mbewe, M. & Ateba, C.N. (2014). Isolation of environmental bacteria from surface and drinking water in Mafikeng, South Africa, and characterisation using their antibiotic resistance profiles. Journal of Pathogens, 2014, 1-11.

Muthuvijayan, V., Gu, J. & Lewis, R.S. (2009). Analysis of functionalised polyethylene terephthalate with immobilized NTPDase and cysteine. Acta Biomaterialia, 5, 3382-3393.

Nersisyan, H.H., Lee, J.H, Son, H.T., Won, C.W. & Maeng, D.Y. (2003). A new and effective chemical reduction method for preparation of nanosized silver powder and colloid dispersion. Materials Research Bulletin, 38, 949-956.

Nghiem, L. D., Schäfer, A.I. & Elimelech, M. (2005). Pharmaceutical retention mechanisms by nanofiltration membranes. Environmental Science & Technology, 39, 7698-7705.

Nie, Y., Qiang, Z., Zhang, H. & Ben, W. (2012). Fate and seasonal variation of endocrine-disrupting chemicals in a sewage treatment plant with A/A/O process. Separation and Purification Technology, 84, 9-15.

Nikolaou, A., Meric, S. & Fatta, D. (2007). Occurrence patterns of pharmaceuticals in water and wastewater environments. Analytical and Bioanalytical Chemistry, 387(4), 1225-1234.

Nissen, K., Stuart, B., Stevens, M. & Baker, A. (2008). Characterisation of animated poly (ethylene terephthalate) surfaces for biomedical applications. Journal of Applied Polymer Science, 107, 2394-2403.

Nordberg, G. & Gerhardsson, L. (1988). Handbook on Toxicity of Inorganic Compounds. Marcell Dekker.
Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout. B.A. Shivaprasad, H.L., Ahmed, S., Chaudhary. M.J., Arshad, M., Mahmood, S., Ali, A. & Khan, A.A. (2004). Diclofenac residues as the cause of vulture population decline in Pakistan. NATURE, doi:10.1038/nature02317, (Accessed from: www.nature.com/nature, published online 28 January 2004).

Oelgemöller, M. & Shvydkiv, O. (2011). Recent Advances in Microflow Photochemistry, Molecules, 16, 7522-7550.

Orata, F. Derivatization Reactions and Reagents for Gas Chromatography Analysis. www.intechopen.com

Ozcam, A.E., Efimenko, K., Jaye, C., Spontak, R.J., Fischer, D.A. & Genzer, J. (2009). Modification of PET surfaces with self-assembled monolayers of organosilane precursors. Journal of Electron Spectroscopy and Related Phenomena, 172, 95-103.

Paíga & Delerue-Matos, C. (2017). Anthropogenic contamination of Portuguese coastal waters during the bathing season: Assessment using caffeine as a chemical marker. Marine Pollution Bulletin, 120, 355-363.

Panyala, N.R., Pena-Mendez, E.M. & Havel, J. (2008). Silver or silver nanoparticles: a hazardous threat to the environment and human health? Journal of Applied Biomedicine, 6, 117-129.

Park, S.Y., Chung, J.W., Chae, Y.K. & Kwak, S. (2013). Amphiphilic thiol functional linker mediated sustainable anti-biofouling ultrafiltration nanocomposite comprising a silver nanoparticles and poly (vinylidene fluoride) membrane. American Chemical Society Applied Materials & Interfaces, 5, 10705-10714.

Patterton, H.G. (2013). Scoping study and research strategy development on currently known and emerging contaminants influencing drinking water quality. WRC Report No. 2093/1/13.

Pavlović, D.M., Ašperger, D., Tolić, D. & Babić, S. (2013). Development and optimization of the determination of pharmaceuticals in water samples by SPE and HPLC with diode-array detection. Journal of Separation Science, 36, 3042-3049.

Perez, S. & Barcelo, D. (2007). Application of advanced MS techniques to analysis and identification of human and microbial metabolites of pharmaceuticals in the aquatic environment. Trends in Analytical Chemistry, 26, 494-514.

Péron, O., Rinnert, E., Lehaitre, M., Crassous, P. & Compère, C. (2009). Detection of polycyclic aromatic hydrocarbon (PAH) compounds in artificial sea-water using surface-enhanced Raman scattering (SERS). Talanta, 79, 199-204.

Petrović, M., Gonzalez, S. & Barcelo, D. (2003). Analysis and removal of emerging contaminants in wastewater and drinking water. Trends in Analytical Chemistry, 22, 685-696.

Pınzaru, S.C., Leopold, P.N. & Kiefer, W. (2004). Identification and characterization of pharmaceuticals using Raman and surface-enhanced Raman scattering. Raman Spectroscopy, 35, 338-346.

Pieczonka, N.P. & Aroca, R.F. (2008). Single molecule analysis by surfaced-enhanced Raman scattering. Chemical Society Reviews, 37, 946-954.

Pillai, Z.S. & Kamat, P.V. (2004). What factors control the size and shape of silver nanoparticles in the citrate ion reduction method? The Journal of Physical Chemistry B, 108, 945-951.

Pinhancos, R., Maass, S. & Ramanathan, D.M. (2011). High-resolution mass spectrometry method for the detection, characterisation and quantitation of pharmaceuticals in water. Journal of Mass Spectrometry, 46, 1175-1181.

Qin, Y., Ji, X., Jing, J., Liu, H., Wu, H. & Yang, W. (2010). Size control over spherical silver nanoparticles by ascorbic acid reduction. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 372, 172-176.

Qu, L., Geng, Y., Bao, Z., Riaz, S. & Li, H. (2016). Silver nanoparticles on cotton swabs for improved surface-enhanced Raman scattering, and its application to the detection of carbaryl. Microchimica Acta, 183, 1307-1313.

Qureshi, A., Singh, N.l., Rakshit, A.K., Singh, F. & Avasthi, D.K (2007). Swift heavy ion induced modification in polyimide films. Surface & Coatings Technology, 201, 8308-8311.

Rao, R., Manu, B. & Kumar, A. (2017). Behavioral, Physical and Biochemical Responses of CyprinusCarpio for Paracetamol Exposure. The International Journal of Emerging Research in Management &Technology. ISSN: Vol6 (2), 2278-9359.

Ratola, N., Cincinelli, A., Alves, A. & Katsoyiannis, A. (2012). Occurrence of organic microcontaminants in the wastewater treatment process. A mini review. Journal of Hazardous Materials, 239, 1-18.

Reichenbächer, M. & Popp, J. (2012). Challenges in molecular structure determination. Berlin: Springer Science & Business Media.

Reznickova, A., Novotna, Z., Kolska, Z. & Svorcik, V. (2014). Immobilization of silver nanoparticles on polyethylene terephthalate. Nanoscale Research Letters, 9, 1-6.

Rivera-Utrilla, J., Sánchez-Polo, M., Ferro-García, M.A., Prados-Joya, G. & Ocampo-Pérez, R. (2013). Pharmaceuticals as emerging contaminants and their removal from water. A review. Chemosphere, 93, 1268-1287.

Rodrigues, D.C., Andrade, G.F.S. & Temperini, M.L. (2013). SERS performance of gold nanotubes obtained by sputtering onto polycarbonate track-etched membranes. Physical Chemistry Chemical Physics, 15, 1169-1176.

Samuel, U. & Guggenbichler, J.P. (2004). Prevention of catheter-related infections: the potential of a new nano-silver impregnation catheter. International Journal of Antimicrobial Agent, 2351, 575-578.

Schaider, L.A., Rudel, R.A., Ackerman, J.M., Dunagan, S.C. & Brody, J.G. (2014). Pharmaceuticals, perfluorosurfactants, and other organic wastewater compounds in public drinking water wells in a shallow sand and gravel aquifer. Science of the Total Environment, 468, 384-393.

Schlücker, S. (2014). Surface-Enhanced Raman spectroscopy: Concepts and chemical applications. Angewandte Chemie International Edition, 53, 4756-4795.

Schmidt, H., Bich Ha, N., Pfannkuche, J., Amann, H., Kronfeldt, H. & Kowalewska, G. (2004). Detection of polynuclear aromatic hydrocarbons (PAHs) in seawater using surface-enhanced Raman scattering (SERS). Marine Pollution Bulletin, 49, 229-234.

Schummer, C., Delhomme, O., Appenzeller, B.M., Wennig, R. & Millet, M. (2009). Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. Talanta, 77, 1473-1482.

Seitz, W., Weber, W., Jiang, J., Lloyd, B., Maier, M., Maier, D. & Schulz, W. (2006). Monitoring of iodinated X-ray contrast media in surface water. Chemosphere 64, 1318-1324.

Sharma, B., Cardinal, M.F., Kleinman, S.L., Greeneltch, N.G., Frontiera, R.R. & Blaber, M.G. (2013). High-performance SERS active materials: Advances and challenges. Material Research Society Bulletin, 38(08), 615-624.

Singh, A., Chaudhary, M. & Sastry, M. (2006). Construction of conductive multilayer films of biogenic triangular gold nanoparticles and their application in chemical vapour sensing. Nanotechnology, 17, 2399-2405.

Sithole, N. (2015). Synthesis of silver nanoparticles and investigating their antimicrobial effects. Published master's thesis. University of the Western Cape, Bellville, South Africa.

Smith, W. (2008). Practical understanding and use of surface enhanced Raman scattering/surface enhanced resonance Raman scattering in chemical and biological analysis. Chemical Society Reviews, 37, 955-964.

Sondi, I., Goia, D. & Matijevic, E. (2003). Preparation of highly concentrated stable dispersions of uniform silver nanoparticles. Journal of Colloid and Interface Science, 260, 75-81.

Sperling, R.A. & Parak, W.J. (2010). Surface modification, functionalisation and bioconjugation of colloidal inorganic nanoparticles. Philosophical Transactions, Series A, Mathematical, Physical, and Engineering Sciences, 368, 1333-1383.

Stackelberg, P.E., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Henderson, A.K. & Reissman, D.B. (2004). Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. Science of the Total Environment, 329, 99-113.

Stiles, P.L., Dieringer, J.A., Shah, N.C. & Van Duyne, R.P. (2008). Surface-Enhanced Raman Spectroscopy. Annual Review of Analytical Chemistry, 1, 601-626.

Strachan, C.J., Rades, T., Gordon, K.C. & Rantanen, J. (2007). Raman spectroscopy for quantitative analysis of pharmaceutical solids. Journal of pharmacy and pharmacology, 59, 179-192.

Subaihi, A., Trivedi, D.K., Hollywood, K.A., Bluett, J., Xu, Y., Muhamadali, H., Ellis, D.I. & Goodacre, R. (2017). Quantitative Online Liquid Chromatography-Surface-Enhanced Raman Scattering (LC-SERS) of Methotrexate and its Major Metabolites. Analytical Chemistry, 89, 6702-6709.

Švorčík, V., Kolská, Z., Kvítek, O., Siegel, J., Řezníčková, A., Řezanka, P. & Záruba, K. (2011). Soft and rigid dithiols and Au nanoparticles grafting on plasma-treated polyethyleneterephthalate. Nanoscale Research Letters, 6, 1-7.

Swartz, C.H., Reddy, S., Benotti, M.J., Yin, H.F., Barber, L.B., Brownawell, B.J. & Rudel, R.A. (2006). Steroid estrogens, nonylphenolethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. Environmental Science and Technology, 40, 4894-4902.

Talbert, J.N. & Goddard, J.M. (2012). Enzymes on material surfaces. Colloids and Surfaces B: Biointerfaces, 93, 8-19.

Taurozzi, J.S. & Tarabara, V.V. (2007). Silver nanoparticle arrays on track etch membrane support as flow-through optical sensors for water quality control. Environmental Engineering Science, 24, 122-137.

Ternes, T.A., Joss, A. & Siegrist, H. (2004). Scrutinizing pharmaceuticals and personal care products in wastewater treatment. Environmental Science and Technology, 38, 392A-399A.

Tijani, J.O., Fatoba, O.O. & Petrik, L.F. (2013). A review of pharmaceuticals and endocrine-disrupting compounds: Sources, effects, removal, and detections. Water, Air & Soil Pollution, 224, 1-29.

Togola, A. & Budzinski, H. (2008). Multi-residue analysis of pharmaceutical compounds in aqueous samples. Journal of Chromatography A, 1177, 150-158.

Vanderford, B.J., Pearson, R.A., Rexing, D.J. & Snyder, S.A. (2003). Analysis of Endocrine Disruptors, Pharmaceuticals, and Personal Care Products in Water Using Liquid Chromatography/Tandem Mass Spectrometry. Analytical Chemistry, 75, 6265-6274.

Vanhaecke, L., Bussche, J.V., Wille, K., Bekaert, K., De Brabander, H.F. (2011). Ultra-high performance liquid chromatography-tandem mass spectrometry in high-throughput confirmation and quantification of 34 anabolic steroids in bovine muscle. Analytica Chimica Acta, 700, 70-77.

Velleman, L., Losic, D. & Shapter, J.G. (2012). The effects of surface functionality positioning on the transport properties of membranes. Journal of Membrane Science, 411, 211-218.

Vesel, A., Junkar, I., Cvelbar, U., Kovac, J. & Mozetic, M. (2008). Surface modification of polyester by oxygen- and nitrogen-plasma treatment. Surface and interface analysis, 40, 1444-1453.

Viviano, G., Valsecchi, S., Polesello, S., Capodaglio, A., Tartari, G. & Salerno, F. (2017). Combined Use of Caffeine and Turbidity to Evaluate the Impact of CSOs on River Water Quality. Water Air Soil Pollution, 228, 330.

Wijnhoven, S., Peijnenburg, W., Herberts, C., Hagens, W., Oomen, A., Heugens, E., Roszek, B., Bisschops, J., Gosens, I., Van de Meent, D., Dekkers, S., Jong, W., Van Zijverden, M., Nne, A., Sips, A. & Re, G. (2009). Nano-silver – A review of available data and knowledge gaps in human and environmental risk assessment, Nanotoxicology, 3, 109-138.

Wille, K., De Brabander, H., Vanhaecke, L., De Wulf, E., Van Caeter, P. & Janssen, C. (2012). Coupled chromatograhic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment. Trends in Analytical Chemistry, 35, 87-108.

Wood, T.P., Duvenage, C.S. & Rohwer, E. (2015). The occurrence of anti-retroviral compounds used for HIV treatment in South African surface water. Environmental Pollution, 199, 235-243.

Xue, L. & Lu, Y. (2013). Electroless copper plating on 1, 2-ethylenediamine grafted poly (ethyleneterepthalate) for the fabrication of flexible copper clad laminate. Journal of Materials Science: Materials in Electronics, 24, 2211-2217.

Yang, M., Cheng, K, Qi, S., Liu, H., Jiang, Y., Jiang, H., Li, J., Chen, K., Zhang, H., Cheng, Z. (2013). Affibody modified and radiolabeled goldeIron oxide heteronanostructures for tumor PET, optical and MR imaging. Biomaterials, 34, 2796-2806.

Yang, X., Shang, C. & Westerhoff, P. (2007). Factors affecting formation of haloacetonitriles, haloketones, chloropicrin and cyanogen halides during chloramination. Water Research, 41, 1193-1200.

Yin, J., Yang, Y., Hu, Z. & Deng, B. (2013). Attachment of silver nanoparticles (AgNPs) onto thin-film composite (TFC) membranes through covalent bonding to reduce membrane biofouling. Journal of Membrane Science, 441, 73-82.

Yu, Y., Wu, L. & Change, A. (2013). Seasonal variation of endocrine disrupting compounds, pharmaceuticals and personal care products in wastewater treatment plants. Science of the Total Environment, 442, 310-316.

Zachhuber, B., Carrillo-Carrión, C., Suaub, B.M. & Lendl, B. (2012). Quantification of DNT isomers by capillary liquid chromatography using at-line SERS detection or multivariate analysis of SERS spectra of DNT isomer mixtures. Raman Spectroscopy, 43, 998-1002.

Zachhuber, B., Ramer, G., Hobro, A., Engelene, H. & Lendl, C.B. (2011). Stand-off Raman spectroscopy: a powerful technique for qualitative and quantitative analysis of inorganic and organic compounds including explosives. Analytical and Bioanalytical Chemistry, 400, 2439-2447.

Zaleski, S., Clark, K.A., Smith, M.M., Eilert, J.Y., Doty, M. & Van Duyne, R.P. (2017). Identification and Quantification of Intravenous Therapy Drugs Using Normal Raman Spectroscopy and Electrochemical Surface-Enhanced Raman Spectroscopy. Analytical Chemistry, 89, 2497-2504.

Zhang, Y. & Sun, J. (2007). A study on the bio-safety for nano-silver as anti-bacterial materials. China Journal of Medical instrument, 31, 35-38.

Zhang, Y., Yu, W., Pei, L., Lai, K. & Rasco, B. & Huang, Y. (2015). Rapid analysis of malachite green and leucomalachite green in fishmuscles with surface-enhanced resonance Raman scattering. Food chemistry, 169, 80-84.

Zhong, L., Mitroshkov, A.V. & Gilmore, T.J. (2016). Analysis of pharmaceutical and personal care compounds in wastewater sludge and aqueous compounds using GCMS/MS. DOI:1013140RG/2.2.10426.80320.

Zorita, S., Boyd, B., Jönsson, S., Yilmaz, E., Svenssona, C., Mathiassona, L. & Bergström, S. (2008). Selective determination of acidic pharmaceuticals in wastewater using molecularly imprinted solid-phase extraction. Analytica Chimica Acta 626, 147-154.

APPENDIX

SERS Costing Calculations

Table 1: Sample preparation cost

Chemical Reagents	LC-MS	GC-MS	SERS
Diclofenac Sodium Salt	R 2 340	R 2 340	R 2 340
Ibuprofen Sodium Salt	R 1 240.32	R 1 240.32	R 1 240.32
Acetaminophen	R 1 882	R 1 882	R 1 882
Methanol HPLC grade	R 473.64	R 473.64	R 473.64
Diethylenetriamine			R 866.40
Methanol HPLC grade			R 473.64
Ethanol Absolute uniVAR			R 1 072.75
Silver Nitrate uniVAR			R 1 865.16
Trisodium Citrate Dihydrate Emaprt A			R 174.08
HCL analytical grade			R 102
Total Cost	R5 936	R5 936	R10 490

Table 2: Solid Phase Extraction

Stage	LC-MS	GC-MS	SERS
Strata-X 33µm polymeric C_{18} reverse phase columns (500 mg/6 mL) which were obtained from Phenomenex	R4206.70	R4206.70	R4206.70
Preconcentration with Methanol	R473.64	R473.64	R473.64
Cleaning with Acetone:Ethylacetate(1:1)	R1 169.70 R896.00	R1 169.70 R896.00	R1 169.70 R896.00
Total costs	R6746.04	R6746.04	R6746.04*

* This cost may not be needed as the TM SERS platform could upconcentrate after pre-separation

Table 3: Analysis cost for each instrument

Analysis	LC-MS	GC-MS	SERS
1st analysis	R 6 919.80	R 5 130.00	R2 800.00
2nd analysis	R 22 200.75		
Total Cost	R29 121.00	R5 130.00	R 2 800.00

Table 4: Instrument cost (Prices obtained March 2019)

Instrument and Accessories	LC-MS	GC-MS	SERS
A Waters UPLC with XEVO TQ MS	R5.8M excl VAT		
Aquity UPLC BEH C18 (2.1 x 100 mm; 1.7 µm particle size) column	R17 580.08		
Acetonitrile 2L	R3 240.00		
Formic Acid 1L	R955.14		
Centrivap (ExcelVap)		R250 000 excl VAT	
Waters Xevo TQ-GC-MS/MS		R3.5M excl VAT	
Integra-Guard (15 m, 0.25 mm ID, 0.25 µm film thickness) capillary column		R15 850.00	
BSTFA 25g		R1 702.00	
Pyridine 100 ml		R662.00	
Espectr R532 Desk top			R246 196.50
PET membrane 1metre			R32 118.19
Total cost	R5 821 775.22	R3 768 214.00	R278 314.69

Table 5: Total cost of the experiment and equipment

Method	LC-MS	GC-MS	SERS
Total Cost	R5 863 578.26	R3 786 026.04	R298 350.73

Table 6: SERS instruments

Instrument	Hand held Raman Analyser RaPort	Espectr R532 Desktop
Cost Price	R269 610.00	R246 196.50

Table 7: Track etched membrane and quartz material comparison as platform for SERS analysis

Materials and Chemical Reagent	Tack etched membrane	Quartz solid surface
PET membrane x 1 metre	R1442.00	
Diethylenetriamine	R 866.40	R 866.40
Methanol HPLC grade	R 473.64	R 473.64
Ethanol Absolute uniVAR	R 1 072.75	R 1 072.75
Silver Nitrate uniVAR	R 1 865.16	R 1 865.16
Trisodium Citrate Dihydrate Emaprt A	R 174.08	R 174.08
HCL analytical grade	R 102.00	R 102.00
Quartz glass surface		R100.00
Ion Beam Sputtering		R7 200.00
Total Cost	R 5996.03	R11 854.03