

**An investigation into silver nanoparticles removal from water during sand
filtration and activated carbon adsorption**

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ABSTRACT

Wastewater treatment plants (WwTP) act as the principle buffer between anthropogenic sources of Silver Nanoparticles (AgNPs) and environmental targets. AgNPs, given their effective anti-microbial properties, have the potential to negatively impact WwTP processes and organisms within the natural environment. A clear understanding of the fate and transport of AgNPs as they pass through WwTPs is crucial in evaluating AgNPs impacts for WwTP process, the natural environment and in the development of a comprehensive environmental risk assessment for AgNPs. The main aim of this thesis was to carry out an analysis on the fate, transport and transformation of AgNPs through WwTP relevant filtration medias in order to understand more about the toxicological implications for both WwTP processes and receiving environments. AgNPs were synthesised in-house, via an in-situ reduction method, which produced a homogeneous dispersion of nanoparticles of average particle diameter 9.98nm, with a standard deviation of 3.11nm. Column studies and adsorption isotherm experiments were conducted to investigate the fate and transport of silver nitrate, AgNPs and bulk silver across media beds of quartz sand and granulated activated carbon (GAC), both chosen for their relevance in wastewater treatment protocols. TEM imaging and EDS analysis was employed to characterise the AgNPs physically and elementally within the column influents and effluents. An original contribution made to the existing knowledge on AgNPs is that in contrast to bulk silver and silver nitrate, uncoated AgNPs were observed to be highly mobile through the quartz sand media. This high mobility was in contrast with the prior expectation that van der Waals forces of attraction between the positively charged AgNPs and the negative

charge of the silica surfaces within the sand bed would lead to some measure of retention within the column matrix. The resulting high mobility of the AgNPs was attributed to particle surface contamination of boride ions originating from the reduction agent used during the synthesis process. This highlights (and reinforces) the importance of better understanding on the implications of the various methods of synthesis and use of capping agents for AgNPs characteristics and the impact this has on fate and transport. AgNPs were also noted to have been significantly altered after their passage through the quartz sand media, with up to 83% of the sample increasing in size, from 9.98nm to an average of 18.26nm and a maximum of 144nm. Particle size measurements were made using the measuring tool available in the GNU Image Manipulation Program (GIMP). This size increase was attributed to the formation of nano-alloy clusters with residual gold and iron compounds, naturally present within the sand bed. In the case of silver-gold alloy clusters, this is expected to exhibit positive implications for future environmental fates of the resulting AgNPs, where the presence of gold in alloy clusters has been observed to significantly deactivate AgNPs silver ion release. In contrast to the sand, it was observed that the GAC was an effective absorber of AgNPs. However, this was observed to be a size dependant relationship, where the GAC was not observed to be effective for adsorption of bulk silver at particle sizes of 300 – 800nm. In this thesis, in addition to the experimental work, a novel, low complexity technique was developed for the detection and quantification of AgNPs in laboratory aqueous solutions. This protocol utilises a laboratory bench top photometer and gave AgNPs concentration results that reliably and accurately reflected that of ICP-MS and ICP-OES results within a detection range of 0.01 and

20mg/L; where the correlation coefficient between the instrument absorbance response and ICP-MS/OES concentration (at 450nm) was R^2 0.994.

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LIST OF ABBREVIATIONS

AgNPs	Silver Nanoparticles
BOD	Biological oxygen demand
COD	Chemical oxygen demand
DFM	Dark Field Microscopy
DI	Deionised water
DLS	Dynamic Light Scattering
DNA	Deoxyribonucleic acid
EBPR	Enhanced biological phosphorus removal
EDS	Energy-dispersive X-ray spectroscopy
EPS	Extracellular polymeric substances
GAC	Granulated activated carbon
GF-AAS	Graphite furnace atomic absorption spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-OES	Inductively coupled plasma optical emission spectrometry
MBR	Membrane bioreactor
nm	Nanometres
RNA	Ribonucleic acid
SPR	Surface plasmon resonance
TEM	Transmission electron microscopy
TSS	Total suspended solids
UV-vis	UV-visible spectroscopy
WwTP	Wastewater Treatment Plant

CHAPTER 1

INTRODUCTION

1.1 Introduction

1.1.1 Nanotechnology

Nanotechnology describes the method and study of manipulating matter on a molecular scale (1 – 100nm); modifying, enhancing or controlling the functional properties and performance of many materials (Peralta-Videa et al. 2011). Since the early 1980's the emergence and rapid surge in this technology has enabled a wide range of new applications and enhanced the performance of many pre-existing technologies. An economic estimate of the global nanomaterials market conducted by Mordor Intelligence LLP for 2015 was set at USD 4,097.17 million, with the expectation for this to increase significantly to USD 11,252.76 million by 2020 (Mordor Intelligence LLP 2016). In addition, the Consumer Products Inventory has recorded a total number of 1814 nanomaterial products active on the global market for 2014, which represents a thirty two fold increase from 2005; of this silver nanoparticles (AgNPs) are the most frequently advertised and used (Vance et al. 2015). While nanotechnology is fundamentally important in the global economy and likely to be more so in the future, concerns have been expressed regarding the potential for environmental and human health implications in the wake of our incomplete knowledge of this emerging technology. It cannot be assumed that these nano variants will behave as their bulk parent material with regards to fate, transport or toxicity. The technologically unique and beneficial properties that nanoparticles are manipulated and manufactured to exhibit, may also be the cause of certain human and environmental health risks. Such features include a large surface area to volume ratio, increased surface reactivity, altered physio-chemical properties (such as changes in melting point or solubility), or

changes in the crystalline structure (Pavlovski et al. 2016; Savolainen et al. 2010). With the increased use of nanomaterials, a new challenge faced by environmental authorities and protection agencies' is the potential impacts of these nanomaterials on the aquatic environment, which will act as the ultimate sink for almost all discharged nanomaterials.

1.1.2 Silver Nanoparticles

Historically, the predominant use over time for silver, is most notably associated with medical and food preservation disciplines as a broad-spectrum antimicrobial agent. It is documented that in Ancient Greece silver was recognised for its' preservation properties and described by the Greek physician Hippocrates (c. 460 – c. 370 BC), as having “beneficial healing and anti-disease properties” (Alexander 2009; Chen & Schluesener 2008). From c. 425 BC onwards the use of silver to preserve water and other food supplies from contamination was recorded (McGillicuddy et al. 2017; Amato et al. 2011) and much later in the nineteenth century C.S.F. Credé famously reported the first scientific published record for the medical use of a 2% silver nitrate solution (as antiseptic eye drops to safeguard against Neonatal conjunctivitis) (Maillard & Hartemann 2013; Schaller & Klaus 2001; Alexander 2009). In the United States colloidal nanosilver, first registered in 1954 under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), became a popular broad spectrum biocidal agent (Nowack et al. 2011; Reidy et al. 2013; McGillicuddy et al. 2017). Other covetable properties of silver, such as its unique optical properties, plus high electrical and thermal conductivity, have ensured its inclusion across a wide range of applications such as photographic

materials, cloud seeding, electroplating, electrical conductors, solder and brazing alloys, paints, jewellery, coins and mirror production (Rosmary A; Faust 1992; Howe et al. 2002; McGillicuddy et al. 2017).

There currently exist several approaches for the synthesis of AgNPs using either chemical, physical, photochemical or biological routes. Though of all the methods available, chemical reductions of a silver salt (such as AgNO_3) are reported as the most common and effective for producing well-defined colloidal AgNPs of 20nm or less in size, commonly favoured by many commercial applications (Natsuki et al. 2015). The choice of reduction agent, and where applicable, the additional use of a stabilising agent (also known as a capping agent) greatly influences the colloidal stability, reactivity, particle shape and size of the resulting nanoparticles; which in turn will influence AgNPs inherent propensity to release Ag^+ ions, exhibit persistent mobility or chemically interact and react with ligands in their immediate environment (Tolaymat et al. 2010).

During the course of the last 20 years the recent boom in nanotechnology has further exploited and enhanced the application of silver across a diverse range of fields (Radniecki et al. 2011a). Of all the nanomaterials currently on the global market, AgNPs in particular have received keen attention for two well defined reasons. Firstly, nano-silver represents one of the most widely used nanomaterials, found in a range of industrial, medical and domestic applications. Secondly silver nanomaterials are arguably one of the few for which there are clear toxicity implications (Marin et al. 2015; Pavlovschi et al. 2016).

1.1.3 Implications for Wastewater Treatment Plants and the Receiving Environment

It is reasonable to assume that AgNPs will end up in the aquatic environment, where the biota is known to be especially sensitive to the toxic effects of silver and nano-silver. A major route for AgNPs into the aquatic environment is via wastewater treatment plants (WwTP). However, our understanding of how AgNPs behave within WwTP environment and of WwTP capabilities to remove AgNPs from wastewater liquors is presently incomplete. Thus there is a concern regarding the wider environmental fates of AgNPs for both WwTPs and the receiving environment. One key aspect of this concern is linked to the known antimicrobial properties of AgNPs which may be expected to have deleterious effects on the functional microbial fauna present WwTPs, which in turn could compromise the biological processes, due to toxicology or by altering the physical properties of the activated sludge's and biofilms.

Known toxicity implications for [bulk] silver in mammals, including humans, is observed in the accumulation of silver in the body leading to a cosmetic disorder known as argyria, that results from the precipitation of insoluble silver salts, such as silver chloride and silver phosphate, in the skin (Chen & Schluesener 2008). However, silver's ability to interact with cell membrane functions and bind with intracellular proteins, Ribonucleic acid (RNA) and Deoxyribonucleic acid (DNA), can lead to the disruption of normal cellular activity, including cellular respiration, eventually leading to cell death. In higher aquatic organisms, aqueous concentrations of silver of 1-5µg/liter have been shown to be lethal to representative species of insects (*Ephemerella grandis*, *Pteronarcy californica*),

daphnids (*Daphnia Magna*), bivalves (*Crassostrea virginia*, *Mercenaria mercenaria*, *Argopecten irradians*), and fish (*Thymallus arcticus*, *Oncorhynchus kisutch*, *Tautoglabrus adspersus*, *pimephales promelas*, *Onchorhynchus mykiss*, *Pseudopleuronectes americanus*, *Paralichthys dentatus*, *Leuciscus leuciscus*) (Nehring 1976; Buhl & Hamilton 1991; Gould & MacInnes 1977; Nelson et al. 1976; Calabrese et al. 1977; Smith & Carson 1977; EPA 1980; Bryan & Langston 1992; Howe et al. 2002). In both freshwater and marine organisms the toxicity effects of silver have been linked to the inhibition of branchial Na^+/K^+ ATPase activities (Webb et al. 2001; Sathya et al. 2012).

The factors that influence the fate and subsequent toxicity of the nano-variant of bulk silver in both WwTP and the environment, include characteristics such as particle size, shape, surface coatings, aggregation and agglomeration states, solubility, surface reactivity and chemical speciation. Common speciations of silver include up to four oxidation states Ag^0 (e.g. metallic silver), Ag^{+1} (e.g. silver nitrate), Ag^{+2} (e.g. silver (II) fluoride), and Ag^{+3} (e.g. tetrafluoroargentate (III)). Where the oxidation state +1 is the most common (Riedel & Kaupp 2009). Silver is known to readily form photosensitive halides (silver salts) which have been usefully employed within the photographic industry. These include silver sulfide, silver chloride, silver bromide, and silver iodide (Hammond 2004). These speciations of silver all exhibit varying properties, characteristics and rates of bioavailability. Where metallic silver is insoluble in water, silver salts (e.g. silver nitrate) tend to be soluble, while AgNPs are typically characterised as a colloidal dispersion exhibiting vary degrees of colloidal stability and dissolution rates, dependant on a range of complex particle and environmental factors (Batchelor-McAuley et al. 2014; Dobias

& Bernier-Latmani 2013). With regards to toxicity, LC₅₀ values (a measure of median lethal concentration) demonstrate an acute toxicity of silver nitrate at (7-day) LC₅₀ 9.1µg/L (Rainbow Trout), whereas in contrast, LC₅₀ values for silver sulphate have been recorded at 20 µg/litre (Daphnids) and rather benign values of 7-day LC₅₀ >100,000 µg/litre for silver thiosulfate, silver chloride and silver sulfide (Atsdr et al. 1990). With regards to the influence of AgNPs surface coatings and particle size, studies represented in the literature report significant toxicological implications for the differing presentations of AgNPs from 48-h LC₅₀ 1.1µg/l for citrate and coffee coated AgNPs, up to 16.7µg/L for uncoated and 176.4µg/l for Sigma Aldrich unknown-coated AgNPs. Ionic silver is generally considered the most lethal at 72-h LC₅₀ 0.5µg/L (Allen et al. 2010; Angel et al. 2013). With regards to the influence of particle size, one study observed a clear relationship between nominal size of AgNPs and the bioaccumulation of Ag in Daphnids. Where over an exposure of 4 hours the influx rates measured for AgNPs were in clear sequence 20 nm > 50 nm > 100 nm (Zhao & Wang 2012). This size dependant toxicity relationship is further supported across other studies where it has been established that AgNPs between 1-10nm typically exhibit greater toxicity than AgNPs of larger dimensions (Hussain et al. 2009; Liang et al. 2010; Morones et al. 2005). This demonstrates the requirement to better understand the physical and chemical behaviour of AgNPs within urban and natural environments; first to understand how the dynamics of how AgNPs react, transform and age as a result of being exposed to complex aqueous matrices, and second to then understand the biological and toxicological consequences of these transformed particles and complexes.

There has been extensive effort in recent years to address the current toxicity concerns for AgNPs in both WwTPs and natural environments. With regards to AgNPs transport through wastewater treatment systems, the predominant focus has been on the potential for adverse toxicological effects for the microbial communities upon which WwTP rely for effective treatment and removal of organic contaminants. An analysis of the literature, provided in Chapter 2, indicates that WwTP performance rates may not be affected for short-term AgNPs exposures, but that long-term chronic exposures may lead to deleterious effects on the diversity of the microbial species present (Jeong et al. 2014; Zhang et al. 2014; Sun et al. 2013; Yang, Li, et al. 2014; Hou et al. 2017). The literature also indicates that while a good proportion of the AgNPs are likely to become associated with the sludge solids; that, this does not unequivocally account for all the AgNPs entering the system and there is still an expectation for AgNPs to be discharged into the receiving environment at an estimated rate of 0.003-0.26 $\mu\text{g/L}$ (Hou et al. 2012; Gottschalk et al. 2009; Wang et al. 2012; Doolette et al. 2013; Lazareva & Keller 2014). As consumer demand and application of AgNP products increases over the coming years, so too will the concentrations of AgNPs entering and exiting WwTP. The motivation for the recent focus on the toxicological impacts of AgNPs for secondary biological treatment processes is clear, however in order to develop a sophisticated fate, transport and toxicity model for AgNPs it is essential to investigate all WwTP compartments, including the primary and tertiary processes of WwTP, which in many cases are realised by methods of filtration and clarification. These processes often represent both the first and last step in a wastewater treatment system and therefore are located at important positions of

opportunity for WwTP to capture AgNPs before either harm is realised within the more central portions of the WwTP or released into the environment.

1.2 Project Aims and Objectives

The aim of this investigation is to evaluate the fate, transport and subsequent toxicity risk of AgNPs through typical filtration medias commonly used within the wastewater treatment industry; employed either as secondary or more commonly as tertiary treatment methods. It endeavours to build upon the work described in other published investigations, which have predominantly focused on various aspects of biological wastewater treatment systems and microbial ecologies, with attention to the toxicological impacts of the AgNPs on wastewater treatment microbes. This research hopes to further expand upon the existing knowledge base of AgNPs transport through wastewater treatment systems by a) focusing on media types that are typical of wastewater treatment techniques such as quartz sand and granulated activated carbon, both common place wastewater filtration methods. An important consideration, where the current literature provided premise is that not all AgNPs are retained within the secondary (typically biological) stages of wastewater treatment processes. While b) assessing the physical and chemical interactions of the nanoparticles within the media environments, with particular reference to how dynamic AgNPs are within these environments and to what extent the AgNPs are affected and transformed as a result of their exposure to the filter medias. Another key consideration, where the bioavailability and toxicity of AgNPs is closely associated with characteristics such as particle size and the chemical speciation.

In achieving the above, this investigation will contribute industrial and environmentally relevant information, pertaining to the mobility of AgNPs through wastewater treatment medias and deliver information on how the AgNPs are affected and transformed both in particle size and chemical characterisation, as a result of their transport through WwTP tertiary systems. This in turn will help inform on their subsequent toxicological fates in the receiving environment. Information such as this will be of beneficial use, contributing to further work developing robust modelling tools that will aid improved wastewater treatment management techniques and AgNPs environmental risk assessments.

In achieving the above aim, the investigation followed four distinct and sequential work phases, a) evaluation of the current challenges of AgNPs risk assessment, including a knowledge gap analysis of the literature, b) the development of a novel photometer protocol for the quantification of AgNPs samples in the laboratory, remote from more sophisticated quantification techniques such as ICP-MS and ICP-OES, c) the running of transport columns and isotherm adsorption experiments for AgNPs, silver nitrate and bulk silver across two different WwTP relevant medias and d) detailed particle size distribution and chemical characterisation analysis of the resulting column and isotherm effluents. Specific goals associated with each task were to:

- i. Develop a literature review paper based upon a critical analysis of the contemporary challenges faced with regards to the potential for environmental risk of AgNPs, both with regards to wastewater treatment systems and subsequently the natural environment (**Chapter 2**).

- ii. Develop a short paper / report detailing the novel photometer protocol employed in the laboratory for the quantification of AgNPs effluent concentrations prior to external ICP-MS / OES results validation (**Chapter 3**).
- iii. Using column and adsorption isotherm studies, evaluate the fate and transport of AgNPs through WwTP relevant medias and in doing so a) determine the mobility of AgNPs through the differing media types, and b) determine the process by which AgNPs may become retained in the media (**Chapter 4**).
- iv. Using TEM and EDS analysis techniques to image and characterize the AgNPs effluents from Chapter 4 for particle size and elemental composition, in order to understand the effect of the media environment on the AgNPs, with the aim for the combination of Chapters 4 and 5 to be developed into a scientific paper (**Chapter 5**).
- v. Utilizing the above data and information to contribute detail regarding the expected / predicted fate of AgNPs through wastewater treatments plants and where relevant, highlight key areas of concern or risk in the natural environment [resulting from WwTP emissions] (**Chapter 6**).

1.3 Outline of thesis structure

This thesis has been divided into six chapters, including that of the introductory chapter, that reflect the study goals and work packages outlined in the previous section.

Chapter 2: Is presented as a paper pending journal submission. This paper details the literature review, which focuses on critically understanding and outlining the contemporary challenges associated with the increased use, and resulting mobility of AgNPs within both the urban wastewater streams and natural environment.

Chapter 3: Also presented as a paper pending submission, this section describes the wet chemical process by which a homogeneous dispersion of yellow colloidal AgNPs of 9.98nm average diameter were synthesised and characterised in the laboratory. While also details the development of a novel yet simple laboratory protocol that was used for the quick determination of AgNPs concentration in laboratory sample solutions prior to ICP-MS/OES analysis. This protocol involved the use a bench top photometer, which following further development and refinement, may prove as a useful support tool for other AgNPs related applications aside from this study.

Chapter 4: Details the silver nitrate, AgNPs and bulk silver transport and adsorption isotherm experiments across media beds of quartz sand and granulated activated carbon. The AgNPs employed in this study were synthesised in the laboratory as described in chapter 2 This section includes detailed methodologies and break through curve analyses and concludes with a discussion regarding AgNPs observed mobility, with potential implications to WwTP and natural environments.

Chapter 5: Using TEM and EDS analysis, this section presents and discusses the particle distribution analysis and AgNPs speciation of the resulting effluents from experimental work in Chapter 4. Where AgNPs were found to be significantly altered as a result of their exposure to the media environments, these changes and alterations in particle characteristics are discussed with relevance to the wider risk for impact within wastewater treatment systems and receiving environments.

Chapter 6: Presents the concluding discussion which combines the data and information provided in previous chapters and brings it together with direct relevance to WwTP treatment methods, with some prediction of environmental risk of resulting effluent emission. The thesis concludes with a summary of the conclusions and recommendations for improving AgNPs environmental risk assessment, by highlighting key areas for future research focus.

1.4 Contribution to Knowledge

- It was observed that AgNPs were highly dynamic and mobile through quartz sand media beds, with circa 100% recovery at point of effluent discharge. It was also found that AgNPs are significantly affected by their transport through the quartz environment, resulting in a high degree of agglomeration and aggregation of up to 83% of the influent mass.
- TEM and EDS data also suggested, though it presently remains unconfirmed, the possible formation of silver alloy nano clusters as a result of both physical and chemical process; to which the range of residual compounds and ligands, such as gold, naturally present within the quartz sand contributed to. In the case of silver-gold nano clusters, it has been observed in the literature that the

presence of the gold in the cluster has a deactivating effect on the AgNPs potential to release silver ions, thereby reducing much of the inherent toxicity potential.

- Activated carbon was an effective adsorbent for AgNPs. Although, with regards to silver in general, it appears that particle size is an important factor in this process; where it was observed that AgNPs of average particle sizes 300-800nm were not adsorbed. It was also noted, that AgNPs exposure to carbon did not have a significant impact on the particles physical or chemical characteristics. Where only 33% of the sample increased in average particle size from 9.98nm up to 13.32nm. This indicates activated carbon as an effective treatment process for the removal of AgNPs, particularly so as a final clarifying process. In addition, it is possible that the adsorption of AgNPs onto the surface of the activated carbon may indeed promote improved wastewater treatment performance, by contributing an additional antimicrobial action to the filtration process.
- As part of the development of this study, a simple, novel laboratory technique was established and employed for the measurement of AgNPs concentration in laboratory influent and effluent samples. This technique utilised a bench top photometer, for which the underlying principle is based upon Beer-Lamberts Law, in which the light absorbance of a solution is considered to be directly proportional to the concentration of the absorbing species in solution and the path length. The accuracy of this method was validated by ICP-MS and ICP-OES and it was found to be effective for laboratory samples between a detection range of 0.1 and 20 mg/L.

CHAPTER 2

**DO SILVER NANOPARTICLES DISCHARGES POSE A SIGNIFICANT RISK TO THE FUNCTIONING
OF WASTEWATER TREATMENT PROCESSES AND THEIR RECEIVING WATERS?**

Paper prepared for Journal submission

**Do silver nanoparticles discharges pose a significant risk to the functioning
of wastewater treatment processes and their receiving waters?**

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Abstract

Wastewater treatment plants (WwTP) act as the main buffer between anthropogenic sources of Silver Nanoparticles (AgNPs) and environmental targets. However, given their anti-microbial properties, AgNPs have the potential to negatively impact the biological treatment processes on which WwTPs rely to treat traditional municipal waste. A better understanding of the fate, interactions, and toxicity of AgNPs as they pass through all functional compartments of WwTPs is required to evaluate AgNPs impacts to waste treatment efficiency, Ag removal, and the effluent receiving environment. In this paper, we carried out a review of existing literature related to the fate of AgNPs in WwTP processes and the toxicological implications for the wastewater microbial communities present. Our analysis suggests that while WwTP performance may not be affected for short term exposures to AgNPs, longer term exposure studies (>50 days) indicate risk for a reduction in the species diversity of the WwTP microbial community. Although effluent emissions of AgNPs from WwTPs are estimated at 0.003-0.26 $\mu\text{g/L}$, a value considered well below the levels expected to induce adverse health effects in aquatic organisms (1-5 $\mu\text{g/L}$), significantly higher levels of AgNPs (0.18-2.01 mg/kg) may accumulate in WwTP sludge solids. Which in turn transfers the greater potential for AgNP environmental risk to the terrestrial environment via land spreading of sludge solid wastes. While, AgNPs in WwTP solids are expected to be present mostly as silver sulphide complexes, which are considered biologically unavailable, this process of sulphurisation may only be partial, thus ensuing the persistence of a presently unknown portion of the AgNPs influent loading into environmental compartments. Based on this review, we summarise that for acute, current exposure data the toxicological risk resulting from AgNPs is low. However,

longer term exposure effects have need for further exploration, where there is still potential for adverse health and population effects for wastewater microbial communities. It is also observed that the terrestrial environment is likely to be a significant receiver of WwTP AgNPs wastes, for which the onward toxicological fates for AgNPs are yet to be fully explored. Due to the continued increase in consumer demand for AgNPs products, AgNPs concentrations in urban waste streams and their receiving waters are likely to increase significantly over the coming years. Further empirical studies are required for proper understanding of the impacts of AgNPs for both WwTP and environmental targets, which should aim to understand the toxicological effects of transformed, aged and recycled AgNPs across all functional sections of a WwTP, including those of filtration and clarification. Such studies can assist in the development of a sophisticated transport and toxicity models for AgNPs, which in turn will provide agency/government and industrial guidance for appropriate legislation and pollution control, and informing on environmental health and toxicology impacts of AgNPs.

Keywords: Silver nanoparticles, wastewater, nanotoxicology, nanotechnology, environment, sulfides

Introduction

Nanotechnology is a rapidly expanding market with important implications for the global economy and socio-economic development. Silver nanoparticles (AgNPs), in particular, have assumed market dominance, in comparison with other nanomaterials, with widespread use in industry, medicine and commercial products

(Sheng, Liu, et al. 2011; Sotiriou & Pratsinis 2011; Yang et al. 2012; Colman et al. 2014; Hou et al. 2012; Radniecki et al. 2011a; Vance et al. 2015). AgNPs are one of the few nanomaterials for which there are known toxicity implications; however, knowledge regarding the environmental impacts of AgNPs remains limited (Siripattanakul-Ratpukdi & Fürhacker 2014; Levard et al. 2013). The perceived risk, and public response in reaction to nanomaterials, could present a significant threat to the safe future development of the nanotechnology industry (Cronholm et al. 2013; Maynard 2006). The industrial and consumer applications and use of AgNPs is forecasted to increase significantly in the near future, and the resulting higher environmental discharge has the potential to impact on various engineered and natural ecosystem processes, including primary productivity, decomposition and the nitrogen cycle (Colman et al. 2014). As such, this presents environmental authorities with the growing challenge of managing the potential environmental risks associated with these discharges (Weinberg et al. 2011).

Wastewater treatment facilities are principally responsible for treating domestic and industrial wastes before release into the receiving environment, thus it is of crucial importance to ensure that existing wastewater treatment operations are not adversely affected by new and novel contaminants, such as AgNPs (Crittenden et al. 2005). Dealing with the presence of AgNPs in water systems becomes even more relevant considering emerging legislation, such as the European Water Framework Directive, which aims to protect the health of river basins as a whole, rather than considering only point impacts (European Community 2000).

While the principal route for AgNPs entry into the aquatic environment is via wastewater treatment facilities (Levard et al. 2012; Siripattanakul-Ratpukdi & Fürhacker 2014; Colman et al. 2014), our understanding of the fate, transport and toxicology of AgNPs within these engineered systems, or the subsequent impacts on the receiving natural ecosystems is incomplete (Hendren et al. 2013; Zhao et al. 2013; von Moos & Slaveykova 2013). Knowledge gained from previous studies on larger sized (often referred to as 'bulk') silver is indicative of environmental fate and impacts, but not necessarily relevant to silver in nano-form(s). (Zhao et al. 2013; Liang, S. a. Bradford, et al. 2013; Nowack & Bucheli 2007; Brar et al. 2010). Projections for Asia, North America and Europe have estimated that 190-410 t/year of AgNPs will pass from industrial and urban environments to WwTP and from there into natural receiving waters and soils, via both liquid and solid wastewater treatment discharges (Siripattanakul-Ratpukdi & Fürhacker 2014). It is predicted that in WwTPs AgNPs will largely be retained in the sludge solids in the form of silver sulfide complexes (Blaser et al. 2008; Kaegi et al. 2013; Doolette et al. 2013; McGillicuddy et al. 2017). Wastewater treatment sludge solids are often used as a fertiliser for agricultural soils, or disposed of in landfill. In both cases AgNPs or aged and / or chemical speciations thereof, are still likely to end up in the aquatic environment (including groundwater sources) via processes of surface run off and soil leaching (Blaser et al. 2008).

One of the main uses of AgNPs in manufactured products is as a bactericide (Marambio-Jones & Hoek 2010), so it is reasonable to expect that AgNPs may affect the functional processes and efficacies of the microbial community in wastewater treatment facilities (Brar et al. 2010). Furthermore, the capability of

treatment processes to effectively remove these nanoparticles from wastewater streams is not well understood or quantified (Brar et al. 2010). In response to these concerns, several regulatory bodies across the UK, Europe and the US are actively supporting significant research programmes on the environmental implications of AgNPs (Maynard et al. 2006). Some of these initiatives include EU-funded projects such as NanoFATE project (NanoFate & Anon 2010); the Transatlantic Initiative for Nanotechnology and the Environment (Tine 2013); the United States Protection Agency led projects for Risk Assessment for Manufactured Nanoparticles Used in Consumer Products; the Consortium for Manufactured Nanomaterial Bioavailability and Environmental Exposure (EPA & Agency 2010; United States Environmental Protection Agency 2010); and the UK based Marina project for managing the risks of nanomaterials (Marina 2013).

This review critically assesses the available knowledge on AgNPs with regard to their transport and reactive fate through the various wastewater treatment processes. We first outline the current state of knowledge on the source and use of AgNPs and also discuss some of the known toxicity implications and controversies of AgNPs. We then assess the available knowledge on the observed fates of AgNPs when exposed to the physical, chemical and biological, processes employed by WwTP and critically assess whether current, and projected environmental discharges of AgNPs pose a concern for aquatic biota and for WwTP operational and management practices.

Wastewater Treatment: Defence at the Frontline

Wastewater treatment facilities mark an important functional barrier between industrial, commercial and consumer use of AgNPs and the receiving environments, both aquatic and terrestrial (Roy et al. 2011). Yet there is reason for concern as to whether AgNPs present a relevant risk to the functional health of WwTPs. Should wastewater treatment processes be impeded in any way, the consequences of ineffective treatment of water could result in a wide range of adverse ecological impacts. If toxic contaminants and / or excessive amounts of nutrients are not removed, the released effluent may affect dissolved oxygen levels in natural waters, which can lead to eutrophication. Other potential outcomes include loss of aquatic life through either chronic or acute toxicity or the bioaccumulation and biomagnification of potential harmful contaminants within the food chain (Howe et al. 2002). The key concern is for AgNPs to impact WwTP biological processes as a result of their known antimicrobial properties.

Silver has been long exploited for its anti-bacterial properties, applied in personal hygiene, food preservation and medicine (Chen & Schluesener 2008). Other major uses of silver have included photographic materials, cloud seeding, coins, mirror production, electroplating, electrical conductors, jewellery, paints, solder and brazing alloys (Rosmarie A. Faust 1992; Howe et al. 2002). Over the last 20 years developments in nanotechnology has resulted in a significant increase in the use of silver across a diverse range of applications (Radniecki et al. 2011a). Commercially available products containing AgNPs include nano-silver impregnated clothing, food packaging, paints and pigments, personal care items, baby care products, prophylactics and home medical care items (Sotiriou &

Pratsinis 2011). Circa 435 products on the global market are known to contain silver nanomaterials, with the expectation for this to increase significantly in the near future due to popular consumer demand (Vance et al. 2015). Between 2005 and 2014 there was a substantial 32 fold increase in commercial products containing nanomaterials (Walters et al. 2014; Vance et al. 2015). Medical applications of nano-silver now include air and water antibacterial filters, surface paints and disinfectants, surface coatings for surgical tools, venous catheters, additives to bone cements and implants, impregnated wound dressings and as part of contraceptive devices (Chaloupka et al. 2010; Martinez-Gutierrez et al. 2010; Chen & Schluesener 2008; Tolaymat et al. 2010). Medical research regarding the application of nano-silver has also expanded into the areas of drug delivery (Cho et al. 2008), gene delivery (Brown et al. 2013), cancer therapy (Zhang et al. 2013; Sankar et al. 2013; Cho et al. 2008), and as effective biosensors (Zhao et al. 2011; Sotiriou & Pratsinis 2011). The use of nano silver in killing infectious microorganisms has received particular attention in the medical profession and a driver for this has been of the prevalence of so called “super-bugs” that have increased resistance to antibiotics (Martinez-Gutierrez et al. 2010; Gunawan et al. 2013; Sotiriou & Pratsinis 2011). It should be recognized however, that the quantities of nanosilver used in most medical applications are relatively small, with an estimated 0.5Moz (million ounces) of silver used by the medical industry (2010), which had been projected to increase to 3Moz by 2015 (GFMS Ltd 2011). Putting this into context, in 2015, the total global demand of silver amounted to 1,170.5 Moz, as reported by The Silver Institute and Thomas Reuters, GFMS (O’Connell et al. 2016). Industrial applications consumed the largest share of this supply at 588.7Moz. The predominant sources of nanosilver entering wastewater treatment

plants are from the manufacturing process and high street consumer items (Whiteley et al. 2011) (Figure 1), where AgNPs are projected to account for roughly 55% of the nano-based market (Walters et al. 2014) and via their wide application are estimated to contribute circa 8.8 tonnes per year of AgNPs into [UK] wastewater treatment systems (Whiteley et al. 2013).

Detecting and quantifying AgNPs in complex environmental matrices, such as wastewater treatment liquors and bio-solids, is a major challenge (Colman et al. 2014), and as a consequence there has been a reliance upon predictive model estimates for concentrates of AgNPs in both municipal wastewaters, and their receiving surface waters. A recent study modeled concentrations of AgNPs ranging between 0.003-0.26 μg /L in effluent discharges and 0.18-2.01mg/L AgNPs in bio-solids waste, based upon market production study estimates for New York, Shanghai and London (Lazareva & Keller 2014). Attempts have been made to validate the models applied to predicting environmental levels of AgNPs with measured concentrates, but the values derived tend not to be nano-specific and can therefore provide only an upper limit for AgNPs concentrations (Holden, Klaessig, Turco, Priester, Rico, Avila-Arias, et al. 2014; Hoque et al. 2012; Johnson et al. 2011; Gottschalk et al. 2013; Lazareva & Keller 2014).

Silver Nanoparticles: Toxicity and Controversy

Silver can exist in a wide variety of speciations which in turn affect its chemistry and reactive behaviour (Navarro et al. 2008; Adams & Kramer 1999; Howe et al. 2002). The bioavailability and toxicity of metals are influenced by their chemical

form. Understanding the speciation of silver is therefore crucial to the understanding of the potential for environmental risk (Levard et al. 2012; Levard et al. 2013). In the 1990's increasing demand for silver in a variety of industrial applications, resulted in an upsurge in research regarding the speciation, toxicity potential and environmental transport of silver (Andren & Armstrong 1999). In 2002, the Concise International Chemical Assessment Document (CICAD) was published under joint sponsorship of the United Nations Environment Programme, the International Labor Organization and the World Health Organization, providing a concise summary on the acquired knowledge (spanning 155 published papers) regarding silver toxicity and its implications for the environment. Along with more recent studies, CICAD constitutes one of the more significant bodies of understanding for any metal regarding hazard and dose-response analyses of exposure (Howe et al. 2002). However, with the technological breakthrough in nanotechnology, the engineered nature of AgNPs presents us with essentially a new contaminant which exhibits properties and characteristics unique to its' nano form; so that the previously understood assumptions for [bulk] silver may no longer be relevant.

The toxicological risk of AgNPs, represents a multiple-factor balance between the target organism (or organ), concentration (dose), the speciation of silver and other influential environmental variables, such as pH, temperature, presence of ligands, to mention but a few. As with any substance, it may be assumed that the effects of AgNPs depend on the dose-time exposure, as well as the surround environmental conditions (Siripattanakul-Ratpukdi & Fürhacker 2014). Extensive consideration has been given to the potential for AgNPs adverse effects on wastewater treatment

systems. Though, controversy exists regarding how the various and numerous particle specific and surrounding environmental factors affect the toxicological impacts of AgNPs. Past research, while extensive, does not yet consider every possible combination of influential variables and parameters. For those that have been investigated, the hormesis (dose-response) relationship and the biochemical mechanisms by which hormesis works are still poorly described and understood (Sheng et al. 2018; Jiao et al. 2014).

The physical and chemical properties of AgNPs that affect toxicity include nanoparticle size, shape and surface coating. AgNPs tend to be highly reactive, with greater potential to release free silver ions due to the increased surface area to volume ratio. Particles that fall below 10nm in size are more able to interact with cellular membranes and other components to affect cellular function and as such are observed to be more toxic to microbial life than larger AgNP presentations (Hussain et al. 2009; Liang et al. 2010; Morones et al. 2005; Choi et al. 2008; Zhang et al. 2008). AgNPs may be synthesized into various different shapes, such as spheres, prisms and rods and each of these shape formations exhibits different active surface facets (Pal et al. 2007; Choi & Hu 2008a; Siripattanakul-Ratpukdi & Fürhacker 2014). AgNPs which exhibited prism / triangular shapes have been found to be the more toxic to microorganisms than other shapes. As reported by Pal et al (2007) that *E. coli* was completely inhibited by the truncated triangular AgNPs with Ag content $10 \mu\text{g L}^{-1}$, while the spherical AgNPs of 500 to $1,000 \mu\text{g L}^{-1}$ caused 100% inhibition for the same culture (Siripattanakul-Ratpukdi & Fürhacker 2014). The use of capping agents have also been observed to have a significant impact on the stability and toxicity of AgNPs where both

polyvinylpyrrolidone (PVP) and citrate are notably the most common agents used and observed across many studies as effective AgNP stabilisers, though the toxicity implications for each are less clear, where some studies have observed PVP to present weaker antimicrobial impacts (Pal et al. 2007; Arnaout & Gunsch 2012) while others report increased potential to release Ag⁺ ions (Dobias & Bernier-Latmani 2013). It is clear, that the effects of size, shape and surface coating for AgNP toxicity and mobility have not yet been comprehensively investigated to ensure that every variable and parameter combination has been considered (Sheng et al. 2018). Debate also exists between whether the AgNPs or their released Ag⁺ ions represent the most significant toxicology risk (Holden, Klaessig, Turco, Priester, Rico, Avila-arias, et al. 2014; Yang, Quensen, et al. 2014; Lok et al. 2007; Choi et al. 2008; Pal et al. 2007).

The environmental conditions as presented by WwTPs are even more various and complex, with a full spectrum of physical, chemical and biological interactions that need to be considered.

Fate and transport of AgNPs within wastewater treatment systems.

WwTP typically comprise of a collective assortment of physical, chemical and biological processes assembled in sequence to ensure optimum treatment performance (Crittenden et al. 2005; Metcalf & Eddy 2003). As AgNPs that have been specifically designed to exhibit strong biocidal properties (Choi et al. 2010; Choi & Hu 2008b; Liang, S. a. Bradford, et al. 2013) they potentially have implications for disrupting processes in WwTPs mediated by the microorganisms required as part of the treatment process. The specific functionalization of AgNPs

engineered to serve their original design purpose may add to the potential problems experienced by WwTPs. These engineered properties may affect the physical and chemical processes of WwTPs either by effecting physical functionality of the system or by reducing the process performance by reducing the chemical interactions that are important for processes such as the precipitation and coagulation of suspended solids materials and also the oxidation, ion exchange and chemical stabilization of effluent sludge's and liquor (Siripattanakul-Ratpukdi & Fürhacker 2014). As an example, coatings that are responsible for keeping nanoparticles in a dispersed state may prolong their life and help maintain them in aqueous solution in the aquatic environment (Brar et al. 2010; Liang, S. a. Bradford, et al. 2013). Conversely, where treatment effectively removes the contaminants by retaining them in the biomass / sludge as nanoparticles or transformed complexes, this may then have environmental implications for terrestrial and agricultural systems when the sludge is applied to the land or used in composting.

AgNPs interactions with physical processes in WwTPs

Research on the transport and behavior of AgNPs through the inorganic [physical] processes of WwTP plants, such as pre-screening, sedimentation, flocculation and filtration, is less extensive than that relating to the biological and biochemical processes. AgNP transport and behavior is greatly influenced by physical characteristics of the AgNPs; characteristics which include the surface coating agents, particle size, and AgNP influent concentration. In addition WwTP conditions such as flow velocity, media particle size and pore size distribution,

mineralogy of the media, the ionic strength of and ligand availability in the influent solution will also affect AgNP transport and transformation (Neukum et al. 2014; Jones & Su 2012; Liang, S. a. Bradford, et al. 2013). Increasingly, in the literature it is becoming apparent that particle size affects AgNPs fate (and toxicity). Smaller particles have a greater volume to surface area ratio leading to increased reactivity and greater potential for silver ion release into the immediate environment. Size, both physical and hydrodynamic, also plays a key role in influencing the mobility and transport of AgNPs through WwTP mediums. Smaller particles may be more mobile through a range of primary physical screening, filtering and sedimentation processes; thereby ensuring the presence of AgNPs in the potentially more susceptible, secondary treatment processes, which tend to rely on biological activity. In support of this Hou et al. (2012) found that >90% of citrate coated AgNPs remained mobile in the wastewater liquor following simulated primary wastewater treatment. Following this, and only after 15-day exposure to aerated sequencing batch reactors (SBR) were the AgNPs completely removed from the liquid phase. This study helps demonstrate the importance of developing a better understanding of the specific sorption, aggregation and settling mechanisms taking place with all wastewater treatment compartments; including those more predominantly associated with physical processes. As in the instance of this study, the suggestion is raised that, despite being characterised by a complex mix of bio-solids, ligands and physical mixing actions, that urban sewer systems, pre-screening and primary clarification techniques, may not represent effective wastewater treatment compartments for the successful removal of the AgNPs from the liquid phase. This is an important consideration for many areas of geographic importance for the mining and industrial processing of silver and AgNPs. Such

locations include Mexico, China, Peru, Australia, Russia, Poland, Bolivia, Chile, United States, Argentina and Canada, each of these countries contributing between 3.7 and 189.5 Moz of silver per annum to the global industry (2015 estimate) (O'Connell et al. 2016). Notable locations for the production of nanosilver occurs in the USA, China, Korea, Taiwan, Iran, Thailand, Germany, Japan, New Zealand, Singapore and the UK. As such a significant proportion of silver and nano silver industrial activity is occurring in locations where environmental protection is poorly developed and / or regulated. In 2009, the United Nations estimated that 90 per cent of all wastewaters in developing countries are disposed untreated, into natural water systems (Roy et al. 2011). In many of these locations WwTPs may have not undergone all of the significant developments which have been experienced in the West, and in many cases may be predominantly relying on processes of primary clarification and / or filtration as the main modes of wastewater treatment. In such areas, the impacts of AgNPs are likely to be greater in the receiving environment than for locations where more advanced modes of treatment now exist.

The use of various capping agents on AgNPs has also been demonstrated to exact an influence on particle mobility by altering the hydrodynamic radius (Stokes Radius), by promoting increased mobility and persistence within porous media environments (Zhang & Zhang 2014; Hou et al. 2017; Liang, S. a Bradford, et al. 2013). Conversely, where particles do get trapped within porous media environments, there may be a risk for recycled or backwashed waters. Typically in WwTP systems these recycled or backwashed waters would be returned to the start of the treatment process. Which serves to highlight the potential risk of aged / recycled AgNPs circulating wastewater treatment systems.

AgNPs interactions with Chemical processes in WwTPs

Valid and robust assessments on fate and behaviour and thus toxicity of AgNPs, requires understanding of the speciation properties of silver. Silver has the potential to exist in 38 isotope forms, 4 oxidation states, and through a predisposition to bind with other available ligands, it can also exist in numerous silver oxides, sulfides, nitrates, halides and thiosulfate complexes (Urcell et al. 1998; Whiteley et al. 2011). Each state will alter and affect basic parameters such as reactivity, mobility, solubility and bioavailability, all of which may be influenced and affected by environmental variables such as pH, temperature, presence and concentration of other chemicals and organic ligands (Mukherjee & Weaver 2010; Whiteley et al. 2011). As such, it is important to understand the chemistry of AgNPs within the complex environment of a wastewater treatment system. The vast majority of AgNPs introduced into a simulated wastewater treatment (activated sludge) environment has been shown to predominantly associate with the bio solids rather than remain in the aqueous phase. Kiser et al. (2010) observed that sorption to activated sludge was a significant removal mechanism for AgNPs, where 97% of uncoated and citrate functionalised AgNPs were removed from the liquor phase via processes of aggregation and sedimentation in the wastewater biomass. Similarly, Yu Yang et al. (2012) recorded that more than 90% of polyvinyl alcohol-coated AgNPs were removed, under anaerobic conditions, from the liquid phase and retained within the bio-sludge. However, partly due to limitations on experimental techniques, uncertainty exists regarding how the AgNPs partition between the bio solid and the aqueous phase, though it is generally accepted that

silver is not thermodynamically stable under typical environmental conditions (Howe et al. 2002). Studies investigating the interaction of bulk silver within WwTP and aquatic environments, have shown that it is most likely associated with suspended particles or present as a thiosulfate complex, colloidal silver complex, colloidal silver chloride, silver sulfide, or soluble organic complexes (Howe et al. 2002). Towards this end, there have been a series of studies, that have described the conditions in which AgNPs form complexes with environmentally available ligands, and sulphidation represents a predominant fate for AgNPs within wastewater systems (Choi et al. 2009; Mühling et al. 2009; Kaegi et al. 2013; Kiser et al. 2010; Yang et al. 2012). A process which has been noted to be strongly dependent on AgNPs particle size and the depth and velocity of the flow field within WwTP (Kaegi et al. 2013). Nano-sized silver sulfide crystals have been identified in the final sewage sludge of a standard municipal WwTP, which resulted from AgNPs interaction with sulfides under reducing conditions (Kim *et al.* (2010). This highlights an interesting path for future exploration, as nano-sized sulfide crystals have been observed to exhibit dissolution rates of at least one order of magnitude higher than that of microcrystals of the same material (Liu et al. 2009). As sulfide minerals and mineral nanoparticles play a key role in heavy metal mobility and release over great distances, it is important to better understand the processes behind silver sulfide reactions and the size dependent reactivity and resulting solubility of the resulting nano and micro scale complexes (Hochella et al. 2005; Tech et al. 2010). These studies all highlight the importance of better understanding nano-silver speciation for determining its toxicological risk. The general overall consensus is ionic silver forms the main toxic component that derives from AgNPs, but rates of dissolution vary across the individual silver

speciation products. Therefore, it can be expected that the rate of dissolution will fundamentally affect the toxicity risk imposed at any given point within the WwTP system (Dams et al. 2011; Choi et al. 2010). This theory is strengthened by the fact that the presence of a capping or coating agent, that reduces the rate of silver ion release, is an effective way to reduce toxicity (Dams et al. 2011; Hou et al. 2012). However, there are data also to indicate that AgNPs also have toxicological effects that relate to the particle itself (Choi et al. 2009; Liang et al. 2010; Liang, S. a. Bradford, et al. 2013; Choi & Hu 2008b; Morones et al. 2005), and this factor should not be overlooked until it is better understood.

AgNPs interactions with Biological processes in WwTP

The microbial communities present in WwTP include a diverse range of biofilms, activated sludges (both aerobic and anaerobic), planktonic mixed cultures and to a lesser extent, some pure cultures of single strains (Sheng, Lui, et al. 2011).

The majority of investigations to date regarding the fate and transport of AgNPs through WwTP have focused upon the interactions between AgNPs and the biological processes relevant for wastewater treatment. This is consistent with the microbial toxicity associated with AgNPs exposure. Considerable progress has been made in recent years to assess the [eco]-toxicity of different nanomaterials in a variety of micro and macro organisms found within the aerobic (and anaerobic) reactors of wastewater treatment facilities. These toxicity studies have extended from those focused on specific bacterial species (Guzman et al. 2012; Janardhanan et al. 2009; Karlsson et al. 2009; Khan et al. 2011; Pal et al. 2007; Dams et al. 2011; Radniecki et al. 2011a); to others assessing community level

effects on planktonic species (heterotroph and / or autotroph) (Choi et al. 2008; Choi et al. 2009; Farkas et al. 2011; Pasricha et al. 2012; Sheng, Liu, et al. 2011; Choi & Hu 2008b; Morones et al. 2005; Choi et al. 2010), and those studies on a mature biofilm (García et al. 2012; Kiser et al. 2010; Benn & Westerhoff 2008; Zhang et al. 2014; Yang, Quensen, et al. 2014; Liang et al. 2010; Sheng, Liu, et al. 2011; Hou et al. 2012; Choi et al. 2010). These studies have described species specific toxicity of AgNPs, identifying species both tolerant and susceptible to the effects of AgNPs.

In these studies it has been established that AgNPs between 1-10nm typically exhibit greater toxicity than AgNPs of larger dimensions (Hussain et al. 2009; Liang et al. 2010; Morones et al. 2005), due to their greater ability to attach and cause disruption to cell membranes, their ability to penetrate the cell, and greater rates of dissolution (Morones et al. 2005). Furthermore, capping agents, also have a strong bearing on AgNPs toxicity. AgNPs have been shown to inhibit bacterial growth, most notably in that of gram negative autotrophs such as *Escherichia coli* (Ahmad et al. 2013; Morones et al. 2005; Sheng, Liu, et al. 2011), either through acute toxicity or by causing interference on certain key cellular activities (Choi et al. 2009; Liang et al. 2010; Farkas et al. 2011). Of these, Nitrifying bacteria which represent a major role as part of the bacterial community responsible for aerobic treatment in WwTPs tend to be highly susceptible to the toxic effects of silver and AgNP (Choi et al. 2009; Jeong et al. 2014; Liang et al. 2010; Radniecki et al. 2011a). In contrast, microorganisms largely responsible for anaerobic treatment, primarily (but not exclusively) heterotrophic, gram-positive, denitrifiers, have been observed to be more tolerant to exposure to AgNP (Sun et al. 2013; Jeong et al. 2014; Sheng, Liu,

et al. 2011; Liang et al. 2010; Radniecki et al. 2011b). Sun et al., (2013) showed that certain filamentous organisms, important in providing structural support and protection to the floc matrix in WwTPs were also susceptible to AgNP exposure.

In the past, there had been a tendency for research to focus on planktonic cultures rather than mature or complete biofilm cultures. This contrasts with that occurring in wastewater treatment systems, where mature biofilms operate, and which are known to behave differently compared to their planktonic counterparts (Sheng, Liu, et al. 2011; Choi et al. 2010). Mature biofilms are ecological communities, formed by a collective series of microbial unit exhibiting mutualistic properties that allow for greater success in the management of resources, environmental control, genetic potential and future population viability (Allison et al. 2000). More recent studies have reported that wastewater microbial populations (typically heterotrophs) that are part of wastewater biofilms are more tolerant to AgNPs (Liang et al. 2010; Sheng, Liu, et al. 2011; Khan et al. 2011; Choi et al. 2010) compared with bacteria species studied individually or as groups of planktonic species (isolated from WwTP). Choi *et al.* (2010) and Sheng & Liu (2011) found that microbes, such as *Escherichia coli*, while individually sensitive to the effects of AgNPs, expressed a greater level of tolerance when exposed as part of a diverse community. Jeong *et al* (2014) found the susceptibility to AgNPs exposure differed for each type of microorganism represented in the biofilm communities with *Thiotrichales* being the most sensitive and that despite community protection, the population diversity can be reduced significantly following long term exposure (>50 days), with implications for functional efficiency with regards to treatment processes and performance (sheng et al 2018)

Studies seeking to understand AgNPs effects on the microbial communities have also done so by monitoring treatment performance indicators, including the effective ability to remove certain (measurable) contaminants, such as nutrients or chemical / biological oxygen demand (Som et al. 2011; Wang et al. 2003; Wang et al. 2012; Hou et al. 2012). Initial studies reported little if any notable long term impact upon treatment performance in response to AgNPs concentrations ranging between 0.2 and 40mg/l (Alito & Gunsch 2014; Wang et al. 2012; Yang et al. 2012; Hou et al. 2012). In these studies, an initial reduction in microbial activity or population was noted, but the trend was for microbial communities and processes to recover quickly following exposure. This has been further supported by observations made by Chen et al., (2013, 2012) who observed that stable AgNPs presented no significant toxicological risk to enhanced biological phosphorus removal (EBPR) however, by comparison, when free Ag ions were introduced into the system in increasing concentrations, they were found to be highly toxic to the EBPR process. This highlights the importance of understanding AgNPs dissolution rates within such systems, as the immediate presence of AgNPs may not in itself present a significant toxicity risk, but following oxidation and dissolution the subsequently released Ag ions may pose a high toxicity risk to the system. In contrast however, Yang et al., (2014a) found that ammonia oxidizing bacterial processes were highly sensitive to exposures to AgNPs at 40ppm, (5-7 day exposures) while that nitrite oxidizing processes were notably more tolerant by comparison. Other similar studies have reported nitrifying bacteria were sensitive to the toxic effects of AgNPs with responses observed at ≤ 1 mg AgNPs/l over long term exposures (50 days / >1 month) (Jeong et al. 2014; Liang et al. 2010). The

current discrepancies in these findings for AgNPs for toxicity to biological treatment microorganisms make it difficult to draw any firm conclusions on the short and long term impacts and potential for risk for WwTP biological treatment processes.

Where microbial and performance related tolerance has been observed this has been attributed to the protective nature of the micro-environments created within mature biofilms and flocs (Park et al. 2013; Liang et al. 2010; Yang et al. 2012; Sun et al. 2013). Within these complex matrices, vulnerable organisms can be sheltered within the more enclosed and thus protected portions of the matrix (Sheng, Liu, et al. 2011). It is also suggested that the bacterial secretion of extracellular polymeric substances (EPS) may provide a permeability barrier through which AgNPs have to penetrate to reach the bacteria when they are part of a biofilm. The EPS forms an important structural component of the biofilm, composed mostly of polysaccharides, proteins, DNA, lipids and humic substances, all of which can affect AgNPs and ionic silver reactivity. Previous work with heavy metals, including copper, lead and zinc have observed that some bacteria will upregulate gene expression of EPS following heavy metal exposure (Xiu et al. 2014; Teitzel & Parsek 2003). More recent studies have shown that this occurs also for AgNPs exposure (Yang et al. 2013; Kang et al. 2014). The resulting increased production of EPS provides a matrix that can trap or precipitate the AgNPs, thus reducing AgNPs bioavailability and providing the microorganisms with an increased level of protection against any adverse effects (Kang et al. 2014). This has also been shown to occur for exposure to bulk silver, where aerobic biodegradation of a photo processing wastewater containing 1.85 mg/L of silver was not found to adversely affect the activated sludge process. In that study the

fate of the majority of the silver was in the sludge solids (Pavlostathis & Maeng 1998; Howe et al. 2002).

Thus, studies on short term exposures (<1 month) to AgNPs in WwTP, tend to indicate no significant adverse impacts on WwTP functioning. It may be a different scenario however for longer term (>1 month) exposures to AgNPs with respect to their effects on wastewater microbial communities. Studies of this nature are still limited and somewhat conflicting in their outcomes. Though the general consensus forming appears to be, that while acute microbial inhibition is often observed following an initial shock load of AgNPs, system recovery (both operational and microbial) usually observed after (Chen et al. 2013; Alito & Gunsch 2014). Some of these studies focus on presenting evidence for tolerance to AgNPs exposure, as measured by treatment performance (Chen et al. 2013; Zhang et al. 2014; Wang et al. 2012), whereas many others are documenting observed changes in microbial community structure and diversity, such as up-regulation of the silver resistance gene *silE* and / or increases in EPS following AgNPs exposure. C. Zhang, Liang, and Hu (2014) investigated the long-term (>60-days) effects of 0.1 mg/L AgNPs on membrane bioreactor (MBR) activated sludge performance. For the first 40 days of the experiment they observed little change in the quality of the resulting effluent or in microbial activity. However following day 41, it was recorded that there had been a 50-fold increase in the silver resistant gene *silE* in the MBR. In a another study, the addition of AgNPs at a concentration 1 and 5 mg/L into bench scale anaerobic membrane bioreactors over the course of 285-days, was found to result in decreased efficiency for phosphorus removal. It was also observed that the microbial community changed at the beginning of the study, but subsequently

stabilized despite persistent exposure (Yuan et al. 2015). However, Jeong et al. (2014) observed, the impact of <1mg/L AgNPs on activated sludge bacteria, where it was evident that the nitrifying bacteria were more sensitive to the presence of the AgNPs than the heterotrophic bacteria; and that following 50 days of exposure the microbial diversity of sequencing batch reactors has decreased notably. Significant reductions in nitrifying bacteria would hinder nitrogen removal and could potentially affect the integrity of the floc structure (Sun et al. 2013; Yang, Quensen, et al. 2014). Data such as these reinforce the requirement for long-term exposure studies to help better understand hormesis effects of AgNPs on biological treatment processes over time scales relevant for the operations of WwTPs.

Research Challenges: Reflecting Realism

The developing literature regarding AgNPs fate and transport is starting to help inform on the impact of AgNPs in the operation of wastewater treatment facilities. Laboratory biofilm simulations of wastewater treatment works provide the most relevant systems for these evaluations. There however remain significant gaps in our knowledge regarding aspects such as the actual release rates of AgNPs into wastewater streams; this also includes their particle specific characteristics. As such, assessing and predicting the resultant physical and chemical transformations of the particles in various WwTP compartments remains a significant challenge (Badireddy et al. 2014; Colman et al. 2014; Levard et al. 2013). Furthermore, environmental risk assessments for AgNPs, need better data on the nano-silver products available on the market and their proportional contributions to AgNPs release into the environment. Such data will allow for more accurate mass loading

calculations (quantity and key characteristics) for AgNPs entering into WwTPs (Hendren et al. 2013).

This is important also for informing the wider community of eco-toxicological studies, ensuring that they are operating within environmentally relevant AgNPs concentrations. A another factor, limiting progress is the fact that our ability to detect and quantify [silver] nanoparticles within natural environmental matrices is highly restricted and this remains one of the crucial priorities for the advancement of our understanding of AgNPs and the subsequent implications for WwTP and receiving environments (Weinberg et al. 2011; Colman et al. 2014).

The association of AgNPs with total suspended solids (TSS), sulfides and other ligands in wastewater (Kaegi et al. 2013), will fundamentally affects toxicological outcome. Where particle transformation and elemental speciation occurs, it is equally important to understand the potential toxicological risks of the transformed complexes, as well as that of the original particles, particularly so where we have learned that the resulting transformation may exhibit strikingly new physical and chemical characteristics from that of the parent product (Levard et al. 2012).

The majority of AgNPs studies have focused upon the impacts of AgNPs [toxicity] for the biological wastewater treatment processes. While this is undoubtedly an important consideration, it is also necessary to consider the fate and transport of AgNPs through all wastewater treatment compartments, individually and sequentially. Firstly, as this will help better inform on how AgNPs react to and are altered by the WwTP environment, which in turn will lead to improved information

regarding more realistic data describing the subsequent toxicity of the transformed and / or aged nanoparticles. Secondly, a better understanding of the ability of the characteristically more physical processes of primary (clarification) and tertiary (filtration) to remove AgNPs from the wastewater liquor will assist in understanding the wider environmental risks of AgNPs for natural receiving environments, particularly so in areas where wastewater treatment protocols are less developed.

To date the majority of research has focused on acute effects of pristine forms of AgNPs, where as in contrast the research activities that have extended their endeavors to focus on aged or environmentally transformed AgNPs or the subsequent agglomerations or chemical speciation's is still relatively limited. With respect to WwTP, it is reasonable to assume that AgNPs will have undergone physical or chemical transformation as the material passes through the WwTP and AgNPs will thus will not enter a WwTP, or the receiving water body in a pristine state. To advance our understanding of AgNPs ecotoxicology in WwTP, we need to better understand how AgNPs are transported through and are affected by the urban drainage environment. This should include time relevant exposure studies which would measure, monitor and record AgNPs physical and chemical changes and include key descriptive parameters such as aggregation states, rates of dissolution and compound speciation.

Currently, there are no conclusive real world examples of the adverse effects of AgNPs release into the environment, but equally very few chronic exposure have been conducted that mimic lifelong exposures. Environmental monitoring for AgNPs is a critical requirement for future risk assessments and environmental

management strategies (Luoma 2008). It should also be recognized that microorganisms can adapt to pollutants including silver and this may reduce the impacts of AgNPs on WwTP. To date there has been little research on the adaptive mechanisms of microorganisms to AgNPs or silver ions (Kang et al. 2014; Xiu et al. 2012; Xiu et al. 2014).

Conclusions

The application of AgNPs provides many commercial and societal benefits, but these benefits could be countered by the potential for risk associated with the properties of AgNPs. Although most studies to date indicate a low risk of adverse effects of AgNPs for acute, current exposures data for long term exposure effects indicates a toxicity risk of AgNPs on wastewater treatment microbial communities, especially so for ammonia-oxidizing species. AgNPs concentrations in urban waste (both liquid and solid) streams and their receiving waters are on the increase increasing the likelihood for harmful effects on organisms in the future. Key knowledge gaps that need addressing for advancing our understanding on the implications of AgNPs discharges on WwTP functioning include:

- More comprehensive data on releases of AgNPs into wastewaters and receiving waters, including validated empirical data for AgNPs concentrations.
- Better understanding of AgNPs transformation and subsequent speciation under relevant wastewater treatment conditions, including the physical and chemical treatment processes.

- Better understanding of the resulting mobility, solubility and bioavailability of the transformed complexes.
- Improved analytical techniques to allow for visualizing AgNPs fate and behavior and interactions in complex environmental samples within a WwTP
- Chronic studies on the effects of AgNPs, including aged, recycled and transformed materials, on environmental representative microbial systems for both WwTPs and natural systems.
- Adoption of standard criteria for nanomaterial risk assessments and particle characterization to allow for rigorous comparisons between studies.

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Figures

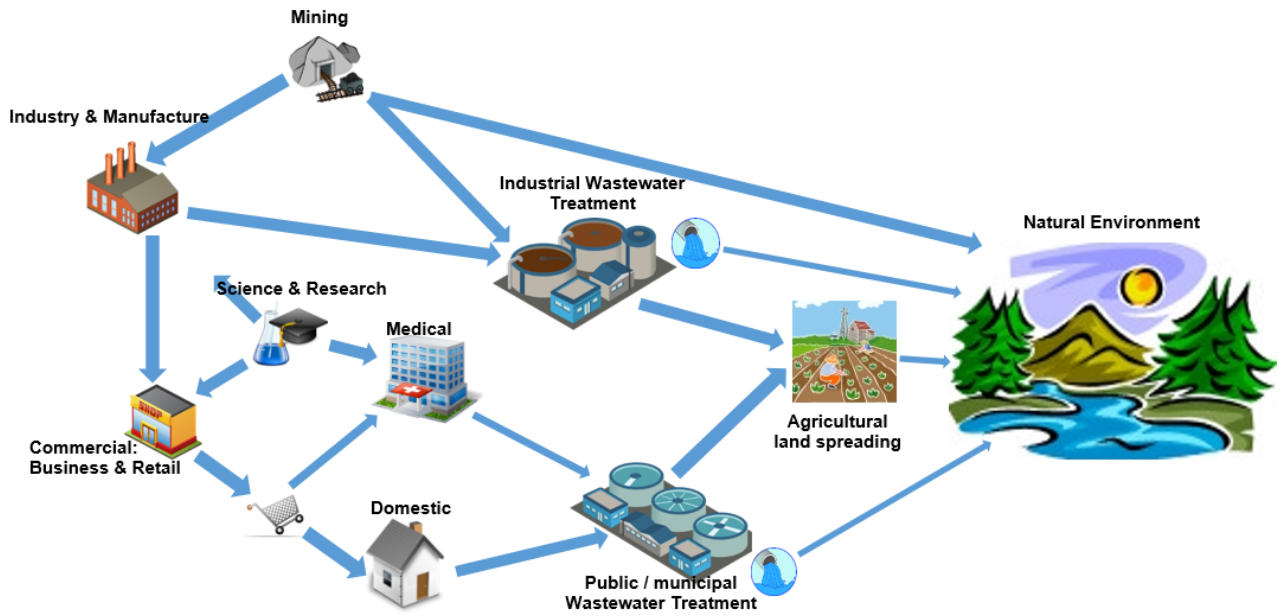


FIGURE 2.2: Pathways of Silver and AgNPs through the urban and natural environment.

CHAPTER 3

DEVELOPMENT OF A NOVEL PHOTOMETER ANALYTICAL PROTOCOL FOR THE QUANTIFICATION OF COLLOIDAL SILVER NANOPARTICLES IN AQUEOUS SOLUTIONS

Paper prepared for Journal submission

**Development of a novel photometer analytical protocol for the quantification
of colloidal silver nanoparticles in aqueous solutions**

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Abstract

The rapid expansion of the nanotechnology industry has many important implications for the global economy. Of these particles, silver nanoparticles (AgNPs) have assumed market dominance. As such, the risk AgNPs present to human and environmental health has become a relevant concern, for which our current knowledge and understanding is incomplete. One of the first priorities that needs to be addressed to ensure we advance our understanding of the risks associated AgNPs, is to develop methods that efficiently detect and quantify silver nanoparticles in complex environmental matrices. Currently we rely upon techniques such as inductively coupled plasma mass spectrometry (ICP-MS); though, these techniques are others like them exhibit their own inherent limitations and are not always readily available or accessible to the user due to factors such as cost and the requirement for a high level of user [technical] training. This paper outlines the wet chemical method used to synthesise a homogeneous colloidal dispersion of yellow AgNPs of 9.98nm in average diameter, and also the development of a low cost, relatively simple technique for the detection and quantification of these AgNPs in aqueous solutions, using a laboratory bench top photometer. The AgNPs samples used to calibrate the technique were synthesised in the laboratory via a process of chemical reduction and all samples were compared for AgNPs concentration using both inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES). The calibration curve derived for photometer absorbance and AgNPs concentration as determined by ICP-MS/OES gave a R^2 value of 0.994. The results from this study demonstrate the feasibility of using such a technique to provide high quality indicative AgNPs concentration values for AgNPs

in aqueous solutions that is AgNPs specific. Pending further development, this method could result in a valuable support technique for AgNPs detection and quantification in aqueous samples useful to laboratory researchers working within the fields of Ag-NP aquatic toxicology.

Keywords: Silver nanoparticles, synthesis, photometer, ICP-MS, nanotechnology,

Introduction

The nanotechnology industry, is rapidly growing, conveying important implications for the global economy. Silver nanoparticles (AgNPs) have assumed market dominance as one of the most widely used nanoparticles spanning industrial and commercial purposes (Sotiriou & Pratsinis 2011; Hou et al. 2012; Sheng, Liu, et al. 2011; Yang et al. 2012; Radniecki et al. 2011a). Nanotechnology describes the material study of manipulating matter on a molecular scale (1 – 100nm), with the aim of modifying and enhancing the functional properties and performance of many materials (Peralta-Videa et al. 2011). This has enabled both the introduction of a wide range of new technical applications and products, and enhanced the performance of many pre-existing technologies (Wang et al. 2012; Marin et al. 2015). The technological developments in nanotechnology are thus proving to be an important contributor to global economies and social security; however, Industry, Policy Makers and Public Stakeholders have expressed concerns regarding the potential environmental and human health implications (Gupta et al. 2015; Maynard et al. 2006). Current knowledge of this new technology regarding particle toxicity and environmental impact, remains incomplete, as also do validated environmental concentrations (Brar et al. 2010; Holden, Klaessig, Turco,

Priester, Rico, Avila-Arias, et al. 2014; Maynard et al. 2006). As consumer demand for AgNPs products rises, so too will the concentration loading of AgNPs in wastewater streams and receiving environmental compartments. As such, new challenges are faced by environmental Policy/Governance authorities and research groups, including safe nanoparticle handling, monitoring and detection and ultimately their human and environmental toxicity implications (Weinberg et al. 2011).

In addressing the above concerns, it is of the highest priority, to establish robust methods that detect, quantify, and characterize silver nanoparticles in complex environmental matrices (Mitrano et al. 2012). More specifically, validated techniques and standards are required to detect and determine relevant concentrations of silver nanoparticles in a variety of complex environmental matrices (Maynard 2006; López-Serrano et al. 2014). This is not just important for the sectors of research and development, but should also be easily accessible by end-users in industry, manufacturing and wastewater treatment; that will ultimately be responsible for monitoring and detecting AgNPs in their wastewater streams and for the Policy/Government enforcing agencies responsible for ensuring that set standards for water quality and environmental safeguarding are maintained.

Presently, several techniques exist that have been adopted and adapted from the existing protocols employed for the detection and quantification of bulk silver. These techniques represent methods and approaches spanning plasma

technology, microscopy and spectroscopy (Figure 3.1). Challenges in quantification techniques for AgNPs include being able to differentiate between chemical and compound speciation and also between varying particle size, particularly so where agglomeration and aggregation within environmental matrices occurs. Commonly used techniques are as described below.

Inductively coupled plasma mass spectrometry (ICP-MS)

This is a highly sensitive technique, typically used for ultra-trace metal determination. It allows for low detection limits and therefore is useful for detecting environmentally relevant concentrations as low as one part in 10^{12} (part per trillion, ppt) (Thomas 2013). It is used for the detection and quantification of both AgNPs and their corresponding ionic form. This method however, relies largely upon solution chemistry, whereby the analyte is digested into soluble form. As a consequence, this technique is not able to differentiate between nanoparticles, ionic form or other dissolved form of the bulk material. Nor is this technique able to distinguish between varying sizes or aggregations of silver nanoparticles (López-Serrano et al. 2014). More recently, the development of single particle (sp)-ICP-MS techniques have allowed for size distribution determination and quantification of AgNPs where the sample is spatially concentrated in advance of analysis, which offers an advantage over traditional characterization methods such as ICP-MS, microscopy, light scattering, and filtration (Mitrano et al. 2012; Hoque et al. 2012; López-Serrano et al. 2014).

Inductively coupled plasma optical emission spectroscopy (ICP-OES)

ICP-OES is regarded for its speed and accuracy, and is capable of detecting elemental concentrations in the μgL^{-1} range. Advantages gained in further developments of this method include being able to detect up to three different varieties of nanoparticles within any one sample and has become a popular technique for *in-vitro* nanotoxicology. It has also been used with success to assess the cellular uptake of nanoparticles of different shapes and sizes. To this end, some of the limitations experienced using ICP-MS have been addressed and overcome (Chithrani & Chan 2007; López-Serrano et al. 2014).

Graphite furnace atomic absorption spectroscopy (GF-AAS)

GF-AAS is an example of spectrometry that employs a graphite-coated furnace to vaporize the sample. As the sample is directly injected into the graphite chamber, minimal sample manipulation or interference is required, thus retaining the integrity of the original sample (Minoia & Caroli 1992). As with the plasma techniques, GF-AAS is capable of handling samples at low concentrations but as with ICP-MS, it is not capable of differentiating between chemical and compound speciation, or varying particle size, unless particle separation techniques have been used in advance (López-Serrano et al. 2014).

In addition to the above, the unique optical properties of silver nanoparticles can be employed to enable methods of detection, characterization and quantification, as we demonstrate in this paper. AgNPs are very efficient at absorbing and scattering light, often referred to as the surface plasmon resonance (SPR), and unlike many

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other compounds, emit a colour that is dependent on the size and the shape of the particle (Mulfinger et al. 2007). Common examples of characterisation techniques utilising the optical properties of AgNPs include UV-visible spectroscopy (UV-vis), Dynamic Light Scattering (DLS) and Dark Field Microscopy (DFM). UV-vis is commonly used to study the structural and morphological properties of silver nanoparticles and is based on either absorption or reflectance spectroscopy within the ultraviolet-visible spectral region. When operated within the range of 300 – 700nm UV-vis is able to return information pertaining to silver nanoparticle size, shape, morphology and stability (López-Serrano et al. 2014). It is also considered possible using the principle of Beer-Lamberts Law (Miller et al. 2009) to also use UV-vis to return information pertaining to concentration (Zook et al. 2011). Dynamic Light Scattering (DLS) is commonly employed to investigate the size distribution of colloidal suspensions of nanoparticles, particle stability and diffusion coefficient which can be related to concentration under steady state conditions. Dark Field Microscopy (DFM) utilizes optical properties for the detection and characterisation of AgNPs within a diverse array of environmental matrices, such as biological tissues (Roh et al. 2009). DFM is well suited for uses involving live and unstained biological samples, such as a smear from a tissue culture or individual, water-borne, single-celled organisms. The main limitation of dark field microscopy is the low light levels seen in the final image. This means the sample must be very strongly illuminated, which can in some cases cause damage to the sample through a process of photo-oxidation (Klein et al. 2015; Grillet et al. 2013).

All of the above described techniques tend to involve high cost, highly complex technical protocols and resources / access to equipment that are not always freely or readily available to all interested users within the associated industrial, research and environmental protection communities. In many cases some notable degree of sample manipulation and preparation is required, thereby reducing representational accuracy while also increasing the labour and time elements of the process. In addition, the above analytical techniques often require a reasonably high level understanding of chemistry, material properties, and optics and therefore a reasonably high level of technical training of the principal user, an attribute expected within a research environment, but not always to same level in other working environment.

This paper first describes the wet chemical reduction method used to synthesis the AgNPs, which was chosen for both its' convenience, but also its' proven success rate in achieving AgNPs of predictable and well-defined colloidal properties (Natsuki et al. 2015). The synthesis process involved a reduction of silver nitrate with sodium borohydride, where deionised water was used as the principal solvent for the entire process. The method was adapted from Mulfinger et al (2007), for where the reduction of the silver salt using sodium borohydride was found to be a reliable method for the production of a homogeneous colloidal dispersion of yellow AgNPs of 12 ± 2 nm particle size and with a plasmon resonance peak of around 400nm.

This paper goes on to describe a novel method, not replicated in the available literature as far as the authors are aware, which was developed in our laboratory. This method provides a fast, minimal sample manipulation, low cost, relatively simple approach for the detection and quantification of AgNPs within solution; utilising both the unique optical properties of silver nanoparticles and low cost, easily accessible piece of laboratory equipment, such as a portable bench top photometer (Palintest Photometer 8000) (Palintest 2003). This is a versatile instrument, which represents a common place support tool often used in the wastewater treatment industry and by field operatives of environmental enforcement agencies. Typically employed for the monitoring of several key wastewater and natural water characteristics, such as nutrient loading, chemical oxygen demand, turbidity, hardness, some metals contamination, among many other parameters that could be mentioned. Advantages of this instrument include that it is easy to use, robust, portable and achieving of results with good levels of accuracy, comparable to those results achieved from a UKAS accredited and/or research laboratory. The underlying principle for this method relies upon Beer-Lamberts Law, whereby the light absorbance of a solution is considered to be directly proportional to the concentration of the absorbing species in the solution and the path length (Miller et al. 2009). This principle is often employed in Spectroscopy techniques with success for AgNPs. We compare the effectiveness of this photometer approach for AgNPs quantification with results obtained from ICP-MS/OES analysis. In assessing the effectiveness of the photometer method, we first established a light absorbance curve for varying aqueous concentrations of AgNPs that was used to confirm the valid assumption and use of Beer-Lamberts Law. Following this, the correlation coefficient between the absorbance data and

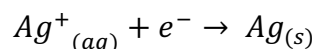
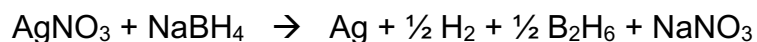
ICP-MS/OES data was determined, and the calibration curve between sample concentration and instrument response described. In the final analysis we discuss the advantages and limitations of this protocol, which we propose as a fast and useful indicative technique for the quantification of silver nanoparticles in aqueous matrices when other methods are not immediately available. We expect this method to be primarily of direct interest to laboratory researchers working within the areas of AgNP aquatic toxicology.

Methodology

Silver nanoparticles synthesis

The chemical reduction method for AgNPs was adapted from Mulfinger, Solomon, Bahadory, *et al.*, 2007. A chemical reduction approach was favoured for convenience and for the established control that it gives over the growth of the AgNPs in order to achieve particles of small size, spherical shape and narrow distribution in diameter (Natsuki *et al.* 2015). All chemicals were sourced through Fisher Scientific Ltd.: 1.0mM Silver nitrate solution prepared from Silver (I) nitrate 99.8+% Specified reagent, and 2.0mM Sodium borohydride solution prepared from Sodium borohydride 98% laboratory reagent. Deionised water was used as the solvent in all cases. All associated glassware (Fisherbrand® Borosilicate) was cleaned using laboratory grade detergent followed by steam autoclave (Autoclave Century 3 non-vacuum 16L, Prestige Medical). The synthesis of silver nanoparticles was achieved by a wet-chemical method, whereby a solution of silver nitrate was reduced by a receiving solution of sodium borohydride. Through the process of dissolving the silver nitrate, the compound was split into a positive silver

ion (Ag^+) and a negative ion (NO_3^-). The free silver ions were then converted into solid silver through the donation of an electron from an electron donor, which in this case was the sodium borohydride.



To control the growth rate and size of the AgNPs and ensure homogenous particles formation, a fast reduction agent such as sodium borohydride was used under ice chilled conditions, with a high rate of agitation and mixing. This method is designed to consistently produce yellow colloidal silver nanoparticles with the size range of $12 \pm 2 \text{nm}$ with a plasmon absorbance around 400nm (Figure 3.2). Important considerations maintained during the process include careful control of the reaction conditions such as stirring intensity, time, temperature, and relative quantities of reagents (both the absolute number of moles of each reactant and well as their relative molarities).

Transmission electron microscopy and Energy-dispersive X-ray spectroscopy

Grids were prepared on copper mesh 400 holey carbon film (Agar Scientific, Electron Technology UK Ltd). 1 drop measured from a microliter pipette (Fisherbrand) was dropped onto the grid and left to dry out thoroughly for 2 days prior to transmission electron microscopy (TEM) analysis. Images were obtained using a 200 kV TEM (JEM-2100 LaB6). Energy-dispersive X-ray spectroscopy (EDS) analysis was performed to confirm the element composition of the sample. Diameter measurements were acquired from the TEM images using the GNU Image Manipulation Program (GIMP). Using the measuring tool provided in the software, first the scale bar for each TEM image was measured and converted into pixels. Particle diameters were then measured in pixels and later converted back into nanometres for analysis. For the purposes of data standardisation, particles were measured under the assumption that all particles were sphere shaped (Horiba Instruments INC. 2010).

Absorbance data

The synthesised sample of silver nanoparticles was split into four batches. The first batch, using volumetric pipettes and glassware, underwent a process of serial x2 dilutions with deionised water, in both cases preceded and followed by sonication (Disintegrator UP100H, Hielscher). 20 ml of each dilution was retained for photometer absorbance and ICP-MS/OES testing. The second batch underwent a similar process of x5 serial dilutions and sonication, and the third batch a series of x10 dilutions and sonication. 20 mls of each dilution phase was retained for each

sample. The last batch underwent a 3 step process of centrifugal concentration to obtain 3 samples of unknown but theoretically higher concentration than the starting sample. The end result was 25 separate samples of varying theoretical concentrations. 10ml of each sample was tested for absorbance using a laboratory bench top Photometer (Palintest Photometer 8000) set at 400nm. The cuvette used was cleaned in laboratory grade detergent, steam autoclaved and finally rinsed with ethanol. Results were recorded against sample ID to determine an absorbance curve. The remaining 10ml of each sample were analysed for silver nanoparticle concentration using the facilities available at The Analytical Research Facility (ARF) at Plymouth University, using both an inductively coupled plasma mass spectrometer (ICP-MS) and inductively coupled plasma optical emission spectrometer (ICP-OES).

Inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectrometry (ICP-OES)

Samples were analysed for silver nanoparticle concentration using a combination of ICP-OES for the suspected higher concentrations and ICP-MS for the lower concentrations. Prior to analysis all samples were placed in a sonic bath for at least 10 minutes and then vortex mixed immediately prior to the analysis. As a separate analysis to ensure data confidence and validation, two samples randomly selected underwent an additional test where 1 mL of sample was removed and mixed with 1 mL of concentrated nitric acid. The mixture was then diluted to 5 mL using deionised water and analysed. Once corrected for the dilution factor, the data for

these two samples was found to be in agreement with those obtained using sonication / vortex mixing. All concentration data was recorded as mg/L.

Results and Discussion

The purpose of this work was to first synthesise a stable yellow colloidal presentation AgNPs of 12 ± 2 nm particle diameters and spherical in shape. Secondly, to develop a fast, low cost, minimal sample manipulation method of determining AgNPs concentration in laboratory samples, where access to more sophisticated analytical techniques such as ICP-MS and ICP-OES were not immediately available. The premise was based upon Beer-Lamberts Law, utilizing the known optical properties of colloidal silver nanoparticles and to correlate this using absorbance values against measure concentrations. The laboratory bench top Photometer provided a key tool by which to achieve this.

AgNPs Synthesis and characterisation

The chemical reduction method employed for this study was found to consistently produce a stable colloidal presentation of AgNPs. Though, during the synthesis process it was noted that the careful control over factors such as reaction time, temperature of the reducing agent, and rate of mixing was imperative in ensuring the consistency of the resulting AgNPs colloidal samples. Unsuccessful synthesis batches were easily and quickly identified due to a rapid change in colour from a clear bright yellow to a dark brown/black of the colloidal suspension, which suggested the formation of an unstable sample in which excessive particle aggregation had occurred. TEM imaging (Figure 3.3) confirms the homogeneous

nature of the spherical silver nanoparticles formed. The average size of individual particles was found to be 9.98nm in diameter, with a standard deviation of 3.43nm, and 3.51nm, for figure 3.3A and 3.3B respectively; with a normal yet narrow size distribution, where kurtosis of the sample was found to be within -0.5 and 0.5. In addition, EDS analysis (Figure 3.4) supported the evidence for the formation of silver nanoparticles against a back drop of copper and carbon present in the sample presentation grid.

Calibration curves.

The absorbance curve (Figure 3.5) plotted for nominal concentration against absorbance obtained for the laboratory synthesised silver nanoparticles at wavelength 400nm, displayed good correlation between the varying nominal concentrations of the samples and the absorbance response of the instrument, with a correlation coefficient of R^2 0.9559. The results obtained at this stage supported the progression of efforts towards establishing a relationship between ICP-MS/OES determined actual concentration and absorbance.

The calibration curve developed for the laboratory synthesised AgNPs (Figure 3.6) presented a strong correlation between ICP-MS/OES determined concentration and instrument absorbance response. Across a dataset of 25 AgNPs samples of varying concentration, the correlation coefficient was R^2 0.994. The dataset shows a limited detection range between 0.01 and 20mg/L (~20 mg/L being the upper limit of the experimental procedure). From reviewing other published works,

relevant concentrations of AgNPs in the environment can be expected to range from 3.0×10^{-6} mg/l to >1mg/l (Holden, Klaessig, Turco, Priester, Rico, Avila-Arias, et al. 2014; Lazareva & Keller 2014; Jeong et al. 2014). These values are based largely upon mathematical assumptions rather than environmental validated data measurements. As such, it is clear that this photometer method, as it currently stands, would not be capable of detecting AgNPs in the lower ranges of expected environmentally relevant concentrations.

The calibration curve for AgNPs can be described using the following curve equation, obtained from figure 3.6:

$$y = (4E - 05)x^3 - 0.0025x^2 + 0.0843x + 0.0058$$

The results from this experiment have supported the use of a Photometer as a fast, low sample manipulation, low cost (zero cost per sample compared to *circa* 10GBP per sample, after initial unit purchasing costs), indicative measure of AgNPs concentration, in the range of 0.01mgL^{-1} to 20mgL^{-1} . Due to the underlying principle by which this method works, concentration data can be assumed to be nanoparticle relevant and as such, free from interference from any ionic silver or other silver speciation present in the sample. The use of ICP-MS and ICP-OES has not been eliminated; however, the results obtained from using the photometer method have proven to be of high value in preliminary decision support, assisting in deciding between ICP-MS and ICP-OES techniques, and giving initial estimates

of silver concentration to assist in the determination of the ICP quantified results. As this system of pre-determination of concentration has progressed and been confirmed for accuracy, confidence in this method has been formed allowing for time and resource savings to be made.

Limitations

The protocol presents several limitations that should be considered before use:

- a) The experimental design is limited by the technical capability of the Photometer, with a range of 1 – 100% Transmittance (T) and a proposed accuracy of $\pm 0.8\%T$. For this method to be of increased value in the detection and quantification of AgNPs at environmental relevant concentrations, a photometer capable of operating across a broader range of optical transmittance values and higher spectral accuracy would be necessary.
- b) This protocol is based upon the premise of light absorption. The AgNPs synthesised samples were created using high grade laboratory reagents, deionised water and well cleaned glassware and equipment and as such were free from and impurities. Such impurities within the sample are likely to influence the absorbance value by affecting light absorption and diffraction. In addition, any impurities present may chemically or physically react with the silver nanoparticles causing aggregation and / or change in chemistry. This in turn may affect the crystalline structure and properties of the silver present, altering the optical properties exhibited. Further experiments in which this method was employed as a preliminary assessment of concentration (prior to ICP-MS/OES) utilised laboratory synthesised effluents. This was found to not

affect the level of accuracy of the method, although in some cases, a slight visible colour difference within the 400nm spectrum was noted which was most likely due to some particle aggregation.

- c) The protocol as described is limited to yellow spherical colloidal silver of average size 9.98nm. It does not include AgNPs of larger or smaller dimensions or of particles of different crystalline structures and arrangements. However, the success achieved within this experiment suggests that it would be possible to further develop this method to be more inclusive of a wider range of AgNPs presentations across a range of different wavelengths outside of the 400nm spectrum; for which the bench top photometer in question is capable of operating at peak wavelengths of $445 \pm 5\text{nm}$, $495 \pm 5\text{nm}$, $555 \pm 5\text{nm}$, $570 \pm 5\text{nm}$, $605 \pm 5\text{nm}$ and $655 \pm 5\text{nm}$.

Concluding remarks

This method has proven effective when an indicative AgNPs concentration value is required. Advantages of this method include speed, minimal sample manipulation prior to testing, the use of relatively low cost instrumentation and a result which can be considered AgNPs specific. In addition, the photometer represents a water quality testing instrument which is readily available on the market and one that is not heavily reliant upon costly consumables or a high level of user training. This method has enabled laboratory users involved in this and subsequent investigations in the decision making process for determining which high end quantification technique (ICP-MS or ICP-OES) would be best appropriate for the

sample and thus has given way to both time, labour and cost saving benefits. This method, following further investigation and development, also presents a plausible low cost (indicative) quantification method for the future use, that could be usefully employed by end-users such as regulatory agencies, industry, wastewater treatment facilities. These specific applications however, are pending further validation and development of the technique to include lower ranges of transmittance to ensure that lower, more environmentally relevant silver nanoparticle concentrations are detectable and also to broaden the scope of the detection method to allow for particles of varying environmentally relevant shapes and sizes.

Future recommendations.

- a) Establishing light absorbance and concentration curves for a range of AgNPs sizes and crystalline structures.
- b) Investigate the influence of commonly employed capping agents on the optical properties of AgNPs.
- c) Calibrating and validating the established concentration curves for a range of environmentally relevant mediums in which AgNPs are suspended / contained.
- d) To investigate the influence of interactions with environmentally relevant ligands upon AgNPs optical properties.

- e) Working within improved and increased ranges for absorbance and transmittance thereby increasing measure accuracy but also the capability to detect and quantify AgNPs at more environmentally relevant concentrations

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Figures

AgNP Quantification Techniques	Plasma Techniques [Quantification]	ICP-MS	Detection of metals at concentrations as low as one part in 10^{13} (part per trillion, ppt)
		(sp)-ICP-MS	As ICP-MS but with the additional potential to detect both the presence of dissolved and particulate forms of an element.
		ICP-OES	An analytical technique used for the detection of trace metals
		GF-ASS	Graphite furnace technique can determine most elements at ppt levels measurable by aspiration atomic absorption in a wide variety of matrices
	Microscopy [Characterisation via Optical Properties]	Dark Field Microscopy	A specialized illumination technique that capitalizes on oblique illumination to enhance contrast in specimens that are not imaged well under normal brightfield illumination conditions
		UV-visible	Routinely employed in analytical chemistry for the quantitative determination of different analytes, such as transition metal ions
		DLS	Used to determine the size distribution profile of small particles in suspension or polymers in solution

FIGURE 3.1: Table outlining the current quantification techniques commonly employed for silver nanoparticles.

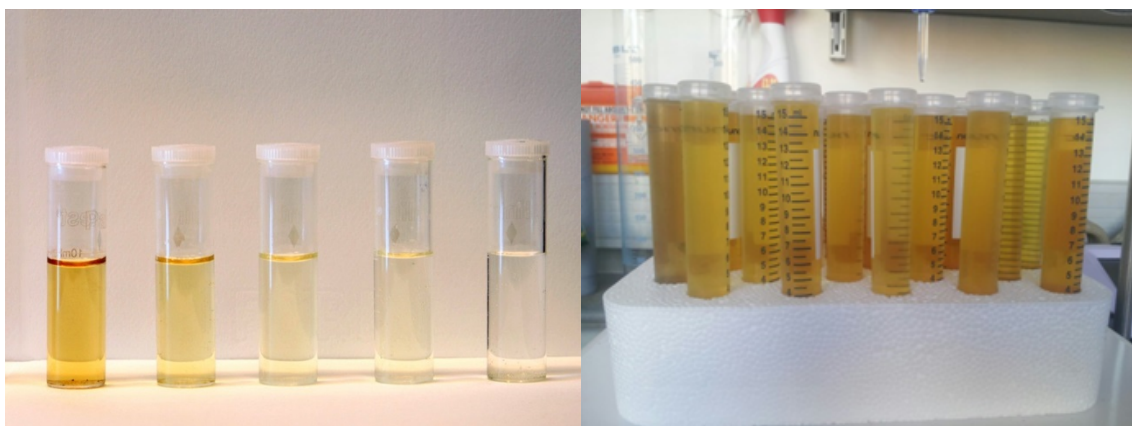


FIGURE 3.2: Laboratory synthesised yellow colloidal silver nanoparticles. Photographic demonstration of visual optical properties. Sample emitting a visible yellow colour.

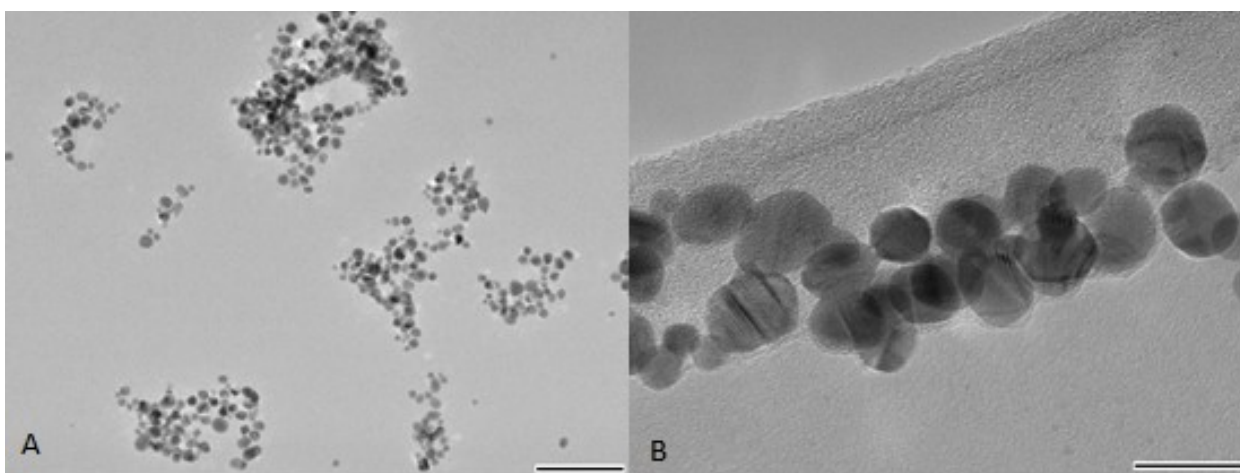


FIGURE 3.3: TEM images showing laboratory synthesised silver nanoparticles at two levels of magnification. In both cases A and B, TEM images confirm the presence of stable AgNPs of 12 ± 2 nm particle diameters and spherical in shape. A: Scale bar 100nm; Direct magnification 40000x. B: Scale bar 20nm; Direct Magnification 250000x.

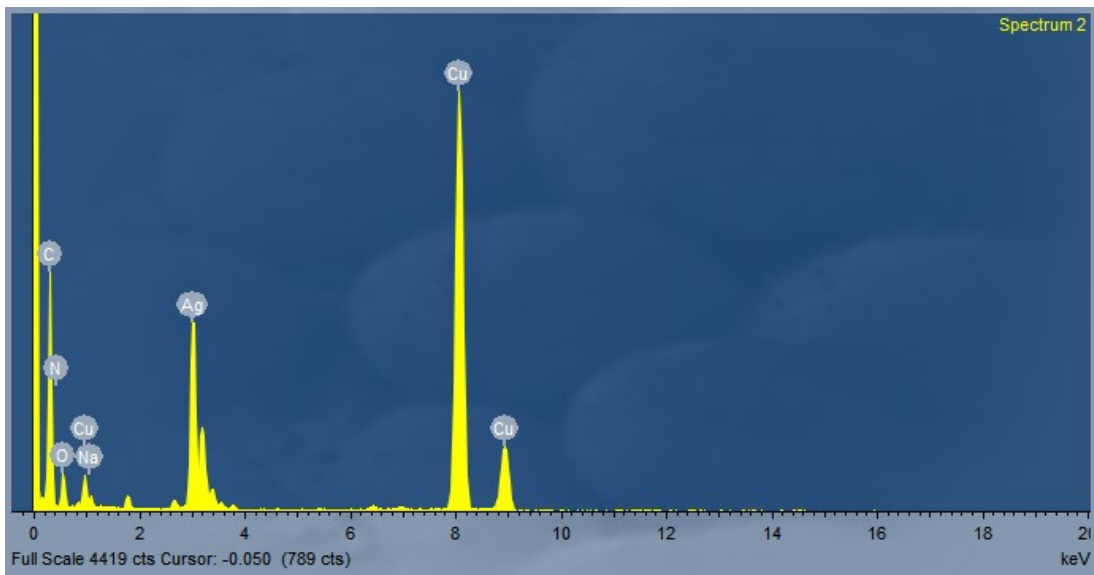


FIGURE 3.4: Energy Dispersive X-Ray Analysis (EDS) analysis for the elemental composition for a sample of the synthesised silver nanoparticles (AgNPs). Provides supporting evidence for AgNPs formation against a back drop of copper and carbon present in the sample preparation.

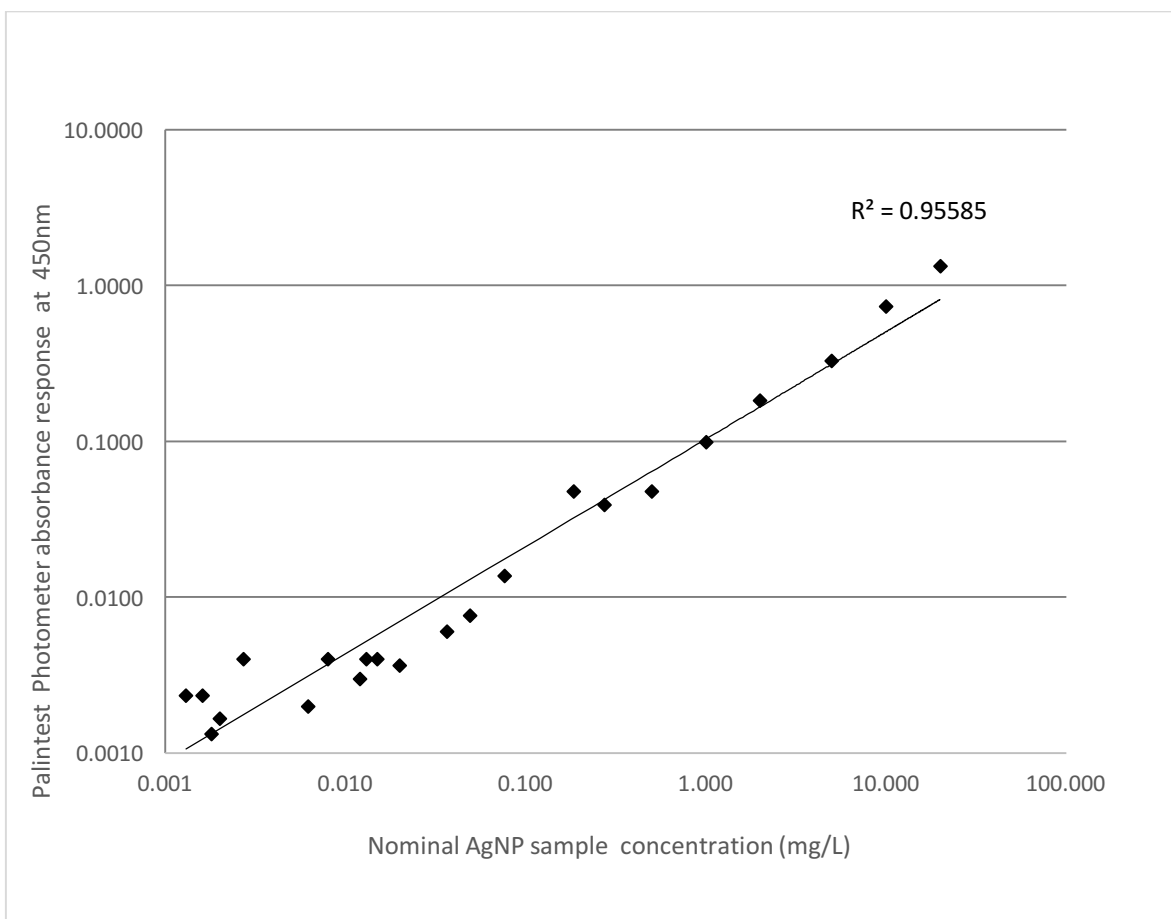


FIGURE 3.5: Simple linear regression analysis, demonstrating a clear relationship between nominal AgNPs sample concentration (mg/L) and light absorbance at wavelength 400nm, using a Palintest Photometer 8000.

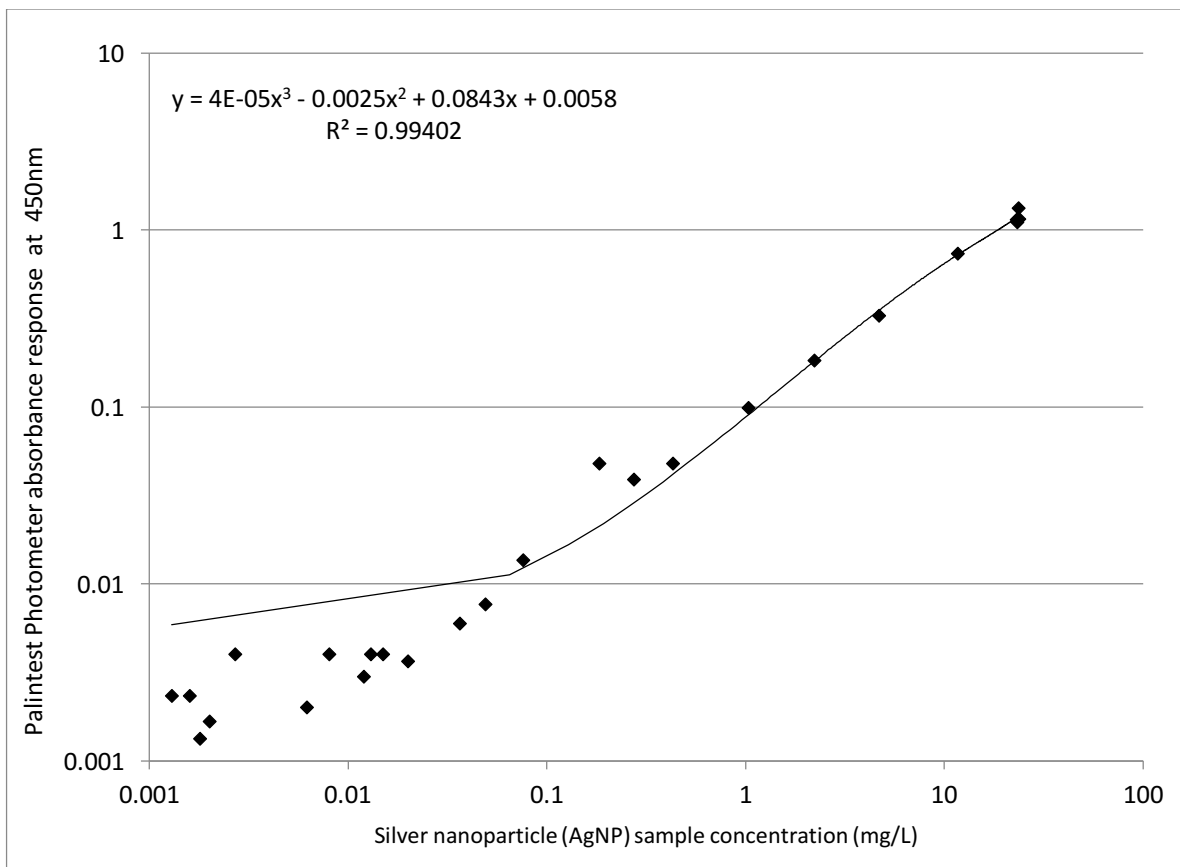


Figure 3.6: Polynomial regression fit to a plot depicting the relationship between the Palintest Photometer instrumental response in absorbance at 450nm to AgNPs sample concentration as determined by ICP-MS and ICP-OES analysis. ICP-MS was employed for AgNPs concentrations assumed to be greater than 10 mg/L and ICP-OES for all AgNPs concentrations assumed to be lower than 10 mg/L.

CHAPTER 4

AGNPs TRANSPORT THROUGH SATURATED SAND AND CARBON COLUMNS

4.1 Introduction

Without a mechanism of treatment in place, the wastewaters resulting from domestic and industrial use would have significant adverse impacts on the natural aquatic environment, via processes of oxygen depletion (due to high BOD content), heavy metal contamination and toxicological effects resulting from pharmaceuticals and other known contaminants of risk. While the regulatory standards and requirements for water quality have over the years become increasingly complex, specific and quantitative, many of the methods we use today are remarkably similar to those methods first employed by ancient civilisations (Mays 2013).

4.1.1 A general history of water filtration

Methods of water treatment, particularly that of filtration, has a long history dating back to the first written recordings of filtration in 2000BC; where it is documented that Ancient Greek and Sanskrit communities well understood the positive impacts of sand, charcoal and gravel filtration techniques, on colour, turbidity and taste of drinking water (Crouch 1993; Koutsoyiannis et al. 2008; Crittenden et al. 2012; Cecen 2011). In 1500 BC the Egyptian's took this a step further, mastering the art of coagulation and sedimentation using chemical additives such as alum, further improving filtration techniques of the day (Pearse 2003). Hippocrates in c. 500 BC developed the Hippocratic sleeve, the first recorded bag filter, effective at removing particulate contaminants from drinking water (and wine) (Sanghavi & Balaji 2013; Mishra & Clarke 2013). After a surge of engineering activity between 300-200BC, resulting in the Roman aqueducts and the invention of the Archimedes screw, there was then a long hiatus in the development of water treatment technology.

However, activity picked back up again in the early 1600s, with Sir Francis Bacon experimenting with the concept of sand filtration as a possible, yet unsuccessful method for desalinization; despite not achieving his original endeavours, his efforts none the less laid the path for future sand filtration techniques (Mishra & Clarke 2013; Mays 2013). Also in the 1600's the first light microscope was developed and in 1676 Van Leeuwenhoek and Robert Hooke first observed and described water borne micro-organisms. With this new revelation, developments in water treatment took on new zeal, as the correlation between many of these microbes and water borne diseases such as cholera and typhoid was established (Gest 2004). In the 1700s the first water filters for domestic application were developed, using filtration medias of wool, sponge and charcoal (Gupta & Mishra 2016; Mays 2013) and in 1804 the first authentic municipal water treatment plant was established in Scotland, UK. This first WwTP was designed by Robert Thom and was primarily based upon the concept of slow sand filtration. In 1890 America took this design one step further and started building large scale sand filters to safeguard public health. However, instead of adopting the slow sand filtration technique they opted for rapid / gravity sand filtration which was proving more effective for high influent volumes, particularly so when preceded by methods of coagulation and sedimentation (Mays 2013). Around this time America was adapting to the introduction of government regulated drinking water standards, which in the 1940s were then also applied to municipal waters. Some 30 years later concerns regarding water quality began to shift from waterborne illnesses, to anthropogenic water pollution such as pesticides, industrial sludge's and effluents, and organic chemicals. Regulations now focused on industrial and domestic wastewaters and the multi-process WwTP more in line with what we recognize today were

established; employing techniques such as aeration, flocculation and activated carbon adsorption (Levin et al. 2002).

Modern day wastewater treatment plants (WwTP) now typically combine a range of wastewater treatment techniques, each designed to remove various contaminants of sewage solids, pathogens, nutrients, toxic chemicals and metals considered dangerous to wildlife and humans alike (DEFRA 2012; Crittenden et al. 2005). The treatment protocol typically provided by WwTP can involve:

- **Preliminary treatment** – for the screening and removal of large solids
- **Primary treatment** – to settle larger suspended, generally organic matter.
- **Secondary treatment** – to biologically breakdown and reduce the residual organic matter.
- **Tertiary treatment** – to address different pollutants using different treatment processes and to provide a method of final clarification.

Processes of filtration are commonly employed in industrial and domestic wastewater treatment as both secondary and tertiary modes of treatment, and can be categorised into two main types. Particulate filters which exclude particle contaminants by size (typically those particles smaller than that which can be dealt with by preliminary and primary treatment processes) , while adsorptive / reactive filters contain contaminants by either a reactive or adsorptive mechanism (Lemley et al. 1995).

4.1.2 Sand Filtration

Sand filters are considered a comparatively, environmentally-friendly wastewater treatment option, which are relatively simple and operationally cost effective. They can be grouped into three main types, slow sand filters, rapid (gravity) sand filters and upward flow sand filters. All three types are extensively used in the water treatment industry around the globe. Rapid sand filters and upward flow filters tend to rely on the pre-treatment of the water with chemical additives to promote coagulation and flocculation, while slow sand filters are able to produce a very high quality of water, free from pathogens without the use of any pre-treatment chemicals. In essence the treatment principle for all three method relies upon grains of sand forming a layer which is penetrable by the wastewater liquor, but will inhibit the flow of larger particles, either by a physical “sieving like” action or via an action of cohesive surface charges, such as van der Waals forces, resulting in physical adsorption (Craft & Eichholz 1970) (Figure 4.1).

Sand filtration is a technology that has been used in modern water treatment systems around the world since the 19th century and is often employed in WwTP where it provides either an early effluent treatment step such as in the case of slow sand filtration (common place in the Western United States and in many developing countries), or more commonly in the case of rapid gravity filtration, providing a final clarifying step in the municipal and industrial treatment process (Vigneswaran et al. 2009; Logsdon et al. 2006; Minnesota Rural Water Association 2009).

Understanding the interactions that take place between AgNPs and porous media environments such as quartz sand is crucial in improving our wider understanding

of the perceived risks to both wastewater treatment systems and their receiving environments; both aquatic and terrestrial. The majority of studies, to date, that have investigated the transport of AgNPs through porous media beds have largely been motivated by desire to investigate the mobility and toxicity impacts of AgNPs in natural environments such as soils and subsurface substrates. Previous studies with quartz sands (Tian et al. 2010; Lin et al. 2011; Song et al. 2011; Lin et al. 2012; Thio et al. 2012; Hou et al. 2017; Yang et al. 2015; Liang, S. a Bradford, et al. 2013; Liang, S. a Bradford, et al. 2013), sandstones (Neukum et al. 2014), loamy sand soil (Liang, S. a Bradford, et al. 2013; Braun et al. 2015), and sandy clay soil (Sagee et al. 2012) have demonstrated that AgNPs transport is highly sensitive to factors such as flow velocity, the surface properties of both AgNPs and porous media environment, the chemical and biochemical composition the of solution, pH value, and the nature of the ligands present. It is also observed that the surface coating employed and/or the presence of organic matter such as surfactant or humic acid can often act to increase the mobility of AgNPs (Tian et al. 2010; Lin et al. 2012; Thio et al. 2012), while higher ionic strength and divalent cations are observed to promote aggregation and retention within the media bed (Zhang et al. 2012; Tolaymat et al. 2010; Lin et al. 2011; Thio et al. 2012). Mobility and retention results from these studies have been variable, most likely a product of the various AgNPs characteristics (including various surface coatings), media environments and solution characteristics. Sagee et al. 2012 observed high rates of mobility for AgNPs through sandy clay soil ranging from 30% to 70% retention within the soil media. It was noted that the mechanisms largely responsible for AgNPs retention were that of physical straining and chemical interactions between the AgNPs and the media surface. Studies that have investigated the influence of

different AgNPs capping agents have observed that PVP has a strong influence in encouraging AgNPs mobility. Hou et al 2017 observed that when coated with PVP, AgNPs were more mobile through quartz sand than bare uncoated AgNPs, where the total release amount of PVP coated AgNPs ranged from 6.37%, 28.42%, 9.5% and 10.03% while that of uncoated AgNPs ranged between 1.54%, 3.28%, 2.58% and 10.36% while exposed to various concentrations of humic acids and alginate. Thus highlighting the important role capping agents may in influencing stability and mobility. Controversy exists however with regards to the role Ionic strength of the solution plays where Zhang and Zhang, 2014 observed that increased AgNPs mobility correlated with an increase in ionic strength in quartz sand columns, while Liang et al 2013 observed that AgNPs mobility decreased with increase ionic strength (in loamy soil).

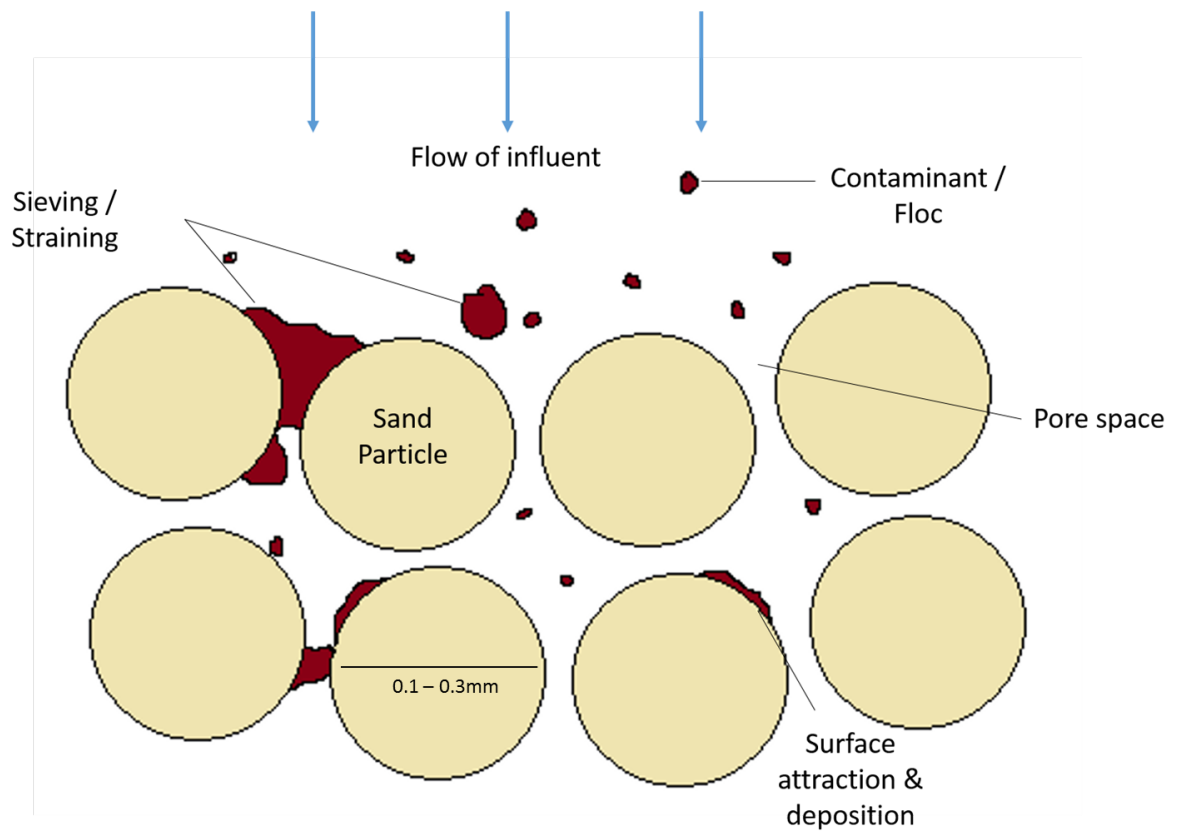


FIGURE 4.1: Schematic diagram illustrating the sieving, straining, surface attraction and deposition action of a basic sand bed.

4.1.3 Activated Carbon

GAC as a powerful adsorbent is one of the most effective media types for removing a wide range of contaminants from industrial and municipal wastewaters; where one typical carbon particle has numerous pores which provide a high surface area to volume ratio (Lemley et al. 1995). The working principle of activated carbon is via a process of adsorption, where contaminants will be attracted to and become trapped (adsorbed) within the various pore spaces and surfaces of the carbon particle, for which the characteristics of the carbon material such as particle and pore size, surface area, surface chemistry, density and hardness will influence this efficiency (Figure 4.2). The characteristics of the contaminate are also important, such as their propensity to leave the water; whereby contaminants that are less water soluble are more likely to be adsorbed (Lemley et al. 1995).

In domestic WwTP activated carbon is useful for removing residuals of pesticides, herbicides, aromatic solvents, chlorinated aromatics and solvents, fuels, alcohols, surfactants, and soluble organic dyes. While compounds having low molecular weight (LMW) and high polarity, such as LMW amines, nitrosamines, glycols, and certain ethers, are not considered to be so effectively adsorbed (Knopp et al. 2016; Cecen 2011). Typical particle sizes that can be adsorbed by GAC range from 0.5 – 50 μ m and contaminant adsorption is optimised by flow control, ensuring the right balance between the volume demand of water being treated and maximum contact time with the filter. Perhaps more commonly recognised for its' use in drinking water treatment plants, GAC is also employed for several other categories of water treatment such as groundwater remediation, landfill leachate, several industrial processes and since the 1960s its popularity with municipal wastewater treatment has been on the increase, as the challenge to meet more stringent regulations and

to remove more complex contaminants from the wastewater liquor becomes more of a contemporary issue (Cecen 2011).

Activated carbon as a media through which to investigate and observed the fate and transport of AgNPs has been far less reported upon in the literature than quartz sand. Activated carbon however does feature in the literature as a substrate onto which AgNPs and various other nanomaterials, may be loaded in order to achieve improved functional qualities including, the improved removal of various dyes and pigments from wastewater effluents (Mehrorang Ghaedi et al. 2012), as a mode of both prevention and treatment of microbial infection (notably E.Coli from drinking water samples) (Tuan et al. 2011), and without the AgNP loading for the recovery of [bulk] silver from synthetic photographic and medical X-ray process effluents, but only following pre-treatment with 0.5 mol/dm^3 sulphuric and nitric acids at $25 \text{ }^\circ\text{C}$, which yielded silver recovery of 98.5% and 95% from sodium and ammonium thiosulfate solutions, respectively (Adani et al. 2005)

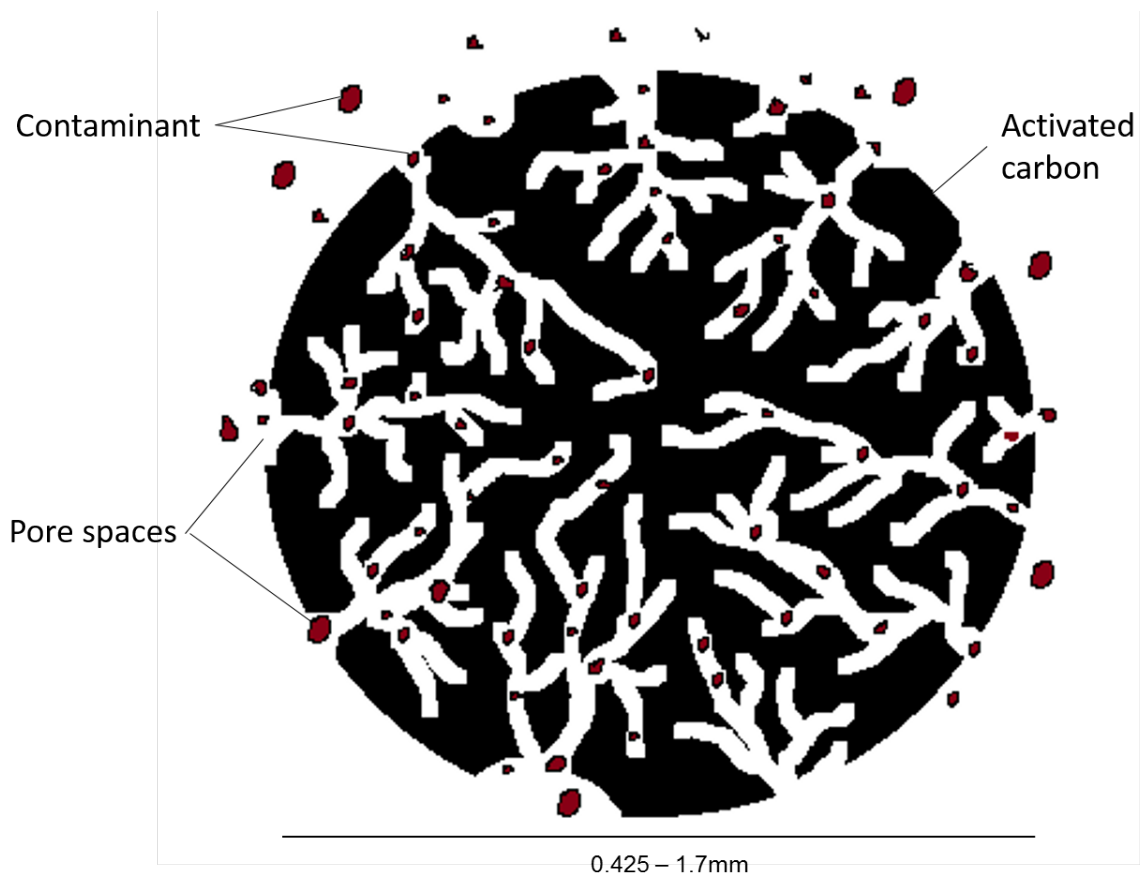


FIGURE 4.2: Schematic diagram adsorption potential and action of an activated carbon particle.

4.1.4 Aims and Objectives

This chapter presents a series of transport and adsorption experiments which evaluate the transport of silver nanoparticles (AgNPs) through quartz sand and activated carbon medias. This is considered an important focal point where current literature investigating the fate of AgNPs through secondary, (biological) wastewater treatment processes, has reported that while a good percentage of AgNPs are trapped in the bio solids via processes of sulphidation, that still circa 25-30% of AgNPs will continue through to final discharge (Kaegi et al. 2011; Gottschalk et al. 2009; Blaser et al. 2008; Hou et al. 2012; Wang et al. 2012; Doolette et al. 2013); currently amounting to an estimated AgNPs effluent emission of 0.003-0.26 $\mu\text{g/L}$ (Lazareva & Keller 2014). As influent concentrations are likely to increase in line with the rising consumer popularity and demand for AgNPs products, so too will future AgNPs influent and effluent concentrations. As such, it remains important to understand the fate and transport of AgNPs through WwTP tertiary filtration systems in order to better understand what risk implications there are for the receiving environment.

In this study, column experiments, considered a key laboratory technique for fate and transport studies through the provision and analysis of break through curves, are employed for the quartz sand media; while isotherm adsorption batch reactor studies were considered more appropriate for the granulated activated carbon (GAC) media.

The choice of sand and activate carbon as filtration beds has been based upon their use in both domestic and industrial wastewater treatment protocols (Crittenden et al. 2005; Brandt et al. 2016). Work packages in this section include:

- a) The preparation of the quartz sand and GAC medias for experimental work, to include the cleaning and drying of prior to use, in order to remove residual unwanted debris and previously adsorbed gases and moisture content.
- b) The preparation of influent solutions, including the synthesis via chemical reduction of the AgNPs (as described in chapter 3) and the calibration of laboratory measuring equipment such as the combination ISE electrode, benchtop pH/ISE/mV/Temp meter and bench top photometer.
- c) The running of laboratory columns for KBr, AgNO₃, AgNPs and bulk silver across quartz sand fixed media beds, obtaining data to support the development of breakthrough curves.
- d) The running of adsorption isotherm batch reactors for AgNO₃, AgNPs and bulk silver, investigating the adsorption potential of GAC for the three silver influent contaminants.
- e) The analysis of quartz sand breakthrough curves, looking for evidence of surface attraction (physical adsorption) forces such as van der Waals between the positively charge silver speciations such as AgNPs and bulk silver and the negatively charge silica surface of the quartz sand.
- f) The analysis of GAC isotherm adsorption in order to determine the adsorption capacity of the GAC for the three differing silver influent contaminations AgNO₃, AgNPs and bulk silver.

4.2 Methods and materials.

Unless otherwise specified, all reagents were sourced from Fisher Scientific Ltd and all solutions were prepared in deionised water. All influent solutions were prepared in Borosilicate glass beakers, which had undergone a double action cleaning programme; washed with Labtek detergent, and rinsed thoroughly with deionised water, followed by a steam autoclave.

4.2.1 Media Preparation

Quartz Sand: The porous media used for the transport experiments was irregular quartz sand (96.2% SiO₂; 1.4% Al₂O₃; 0.1% Fe₂O₃; 0.04% CaO; 0.04% Na₂O; 0.02% MgO), with particle sizes ranging from 0.1 – 0.3mm (Specialist Aggregates Ltd, UK) (Table 4.1). The procedure for cleaning the quartz sand involved first soaking in 0.01M HCL overnight. This was followed by multiple rinsing of the sand in deionised water until both the supernatant ran clear and the pH had stabilised at a neutral position. After this the sand was baked in the oven at 280°C for four hours. The cleaned sand was then transferred into clean plastic containers with lids until required for use.

Activated Carbon: Granular (coconut) activated carbon (GAC), purchased from Industrial Water Equipment Ltd with particle sizes ranging from 0.425 – 1.7mm was used in this study (Table 4.1). Prior to use, the carbon was triple rinsed in deionised water to wash away any particulates and impurities and then dried in the oven at 280°C for four hours to remove adsorbed gases and moisture content. This

preparation was timed to coincide with planned experimental work so that no storage of cleaned carbon was required.

	Quartz Sand	GAC	Unit
Particle sizes	0.1 - 0.3	0.425 - 1.7	<i>mm</i>
Relative density	17	28 - 33	<i>lb/cubic foot</i>
Hardness	7	3	<i>Mohs</i>
Specific gravity	2.65	08 - 2.1	<i>g/cm³</i>
Density	1.6	0.43	<i>g ml/L</i>
pH aqueous phase	6 - 7.5	10	
Surface area		950 – 1075	<i>m²g⁻¹</i>
Pore volume	0.35	0.68	<i>ml g⁻¹</i>

TABLE 4.3: Table of experimental quartz sand and activated carbon media properties and parameters.

4.2.2 Influent Solutions

Bromide conservative tracer: KBr [aq] stock solution (100ppm) was prepared by dissolving 1.4893 g in one litre of water to obtain a target concentration of 1 g L⁻¹ of Br-. Then, following the process of serial dilution this was diluted with deionised water to 100mg L⁻¹.

Silver Nitrate: AgNO₃ [aq] stock solution (100ppm) was prepared by dissolving 1.5748 g of AgNO₃ in one litre of deionised water, to obtain a target concentration

of 1 g L^{-1} of Ag^+ . Then, following the process of serial dilution this was diluted with deionised water to 100 mg L^{-1} .

Silver Nanoparticles: The AgNPs (average 9.98nm diameter) were synthesised as described in Chapter 3. Influent solutions were then prepared to a concentration of 10 and 20 mg L^{-1} . Prior to use AgNPs solutions were sonicated with an ultrasonic processor UP100H (100 watts, 30kHz) for 30 minutes.

Bulk Silver: Bulk Silver 300-800nm sourced from the Facility for Environmental Nanoparticle Analysis and Characterisation (FENAC), Birmingham. Influent solutions were prepared to a concentration of 20 mg L^{-1} . Prior to use, bulk silver solutions were sonicated with an ultrasonic processor UP100H (100 watts, 30kHz) for 30 minutes, and then continually agitated with the use of an orbital mixing plate during the influent feeding process.

4.2.3 Transport experiments

Quartz Sand: Cylindrical Acrylic columns, 6cm in diameter and 30cm in length, were wet packed with cleaned quartz sand at incremental depths of approximately 4cm. At either end of each column, one layer of glass beads (4mm diameter) and a layer #60 mesh stainless steel (sand facing) was installed (Figure 4.3). This was to prevent the loss of sand from the column and to allow for the incoming and outgoing flow to evenly distribute / normalise. The porosity of the packed sand was approximately 0.35 (35%).



FIGURE 4.3: Photographic image showing the set up of the quartz sand columns prior to packing. One layer of glass beads and a layer #60 mesh stainless steel installed at the bottom and repeated again (in reverse) at the top of each column to ensure an even distribution of flow.

The flow through the packed bed can be described by the Ergun Equation.

$$\frac{\Delta p}{L} = \frac{150(1 - \varepsilon)^3 u_o}{\varepsilon^3 d_p^3} + \frac{1.75(1 - \varepsilon) p u_o^3}{\varepsilon^3 d_p}$$

Where Δp represents the pressure drop, L is the height of the media bed, μ is the fluid viscosity, ε is the void space in the media bed, u_o is the fluid superficial velocity, d_p is the particle diameter and p is the density of the fluid.

Bromide was used as a soluble conservative tracer. Following saturation of the columns and washing with four pore volumes of deionised water, a 300ml pulse of 100ppm bromide (as KBr) was pumped across the columns using a peristaltic pump (Watson Marlow Peristaltic Pump 323S) set at 90rpm. The direction of flow across the column length was vertical and against gravity (Figure 4.4). Outflow samples were collected every five minutes for testing and analysis including silver concentration (mg/L), pH, temperature (°C) and sample volume (ml).

Following the conservative tracer transport run, the columns were washed through with eight pore volumes of deionised water. A 300ml pulse of silver (AgNO_3 100ppm; AgNPs 10 and 20ppm, bulk silver 20ppm) and was pumped across the columns abiding by the same pump and flow conditions as mentioned above. Outflow samples were again collected every five minutes for testing and analysis. The duration of the transport runs varied between 130 and 140 minutes depending on the influent contaminant. Where 170 minutes had been observed as ample time for the bromide conservative tracer to enter and exit the system.

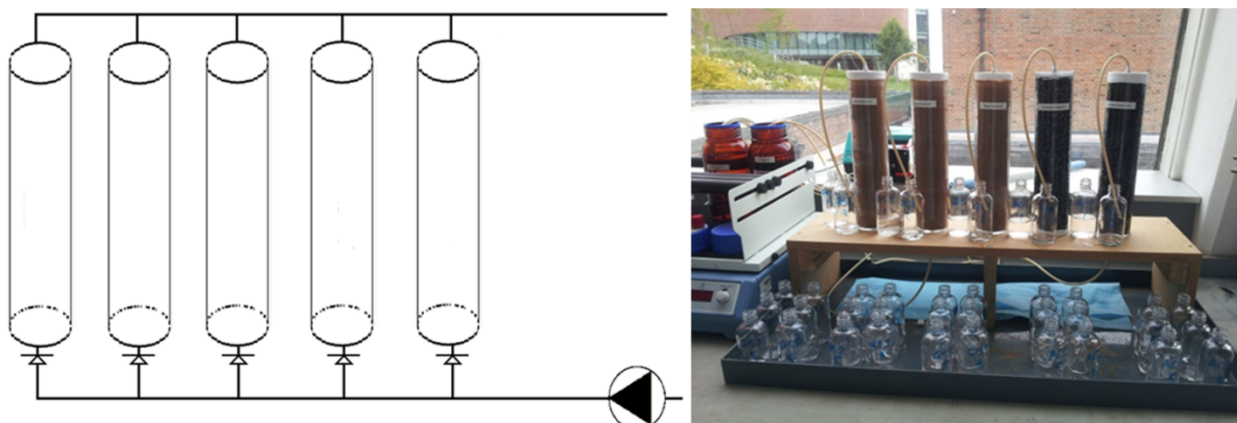


FIGURE 4.4: Schematic diagram and photographic image showing the set up of the quartz sand transport columns.

The silver (AgNO_3 , AgNPs, bulk silver) transport run was immediately followed by a second 300ml pulse of 100ppm bromide (as KBr). Pump and flow conditions remained the same. This final bromide run was conducted to confirm the presence of any residual contaminant in remaining within the system via an expectation for a chemical reaction between the potassium bromide and the silver (nitrate, nanoparticle) to form the halide silver bromide (an insoluble salt). For continuity, this step was repeated for bulk silver columns, though the halide reaction was not expected in this particular case due to the insolubility and therefore reduced availability of the bulk silver. Outflow samples were collected and treated the same as previously described.

Granulated activated carbon: Column experimental runs as with the quartz sand were abandoned for GAC due to length of time required to achieve contaminant

breakthrough. In its stead, GAC adsorption isotherms studies were identified as more appropriate. GAC isotherms are useful for determining the ability of GAC to remove a specific contaminant. The adsorption isotherm describes the equilibrium relationship between the adsorbate and the adsorbent. One of the most common mathematical expressions used to describe the adsorption isotherm is the non-linear Freundlich equation.

$$q_e = KC_e^{1/n}$$

Where q_e is the equilibrium loading on the GAC, K is the adsorption capacity at unit concentration, C_e represents the equilibrium concentration in the water and $1/n$ is the strength of adsorption.

The isotherms were run with up to 18 batch reactors consisting of glass beakers in parallel. Each beaker contained the same concentration (10mg/L) and volume (200ml) of contaminant solution. Each flask was dosed with a different amount of carbon (1 up to 14 and 18g) and gently mixed until equilibrium between phases was reached. C_e was measured, and the GAC loading was calculated, assuming conservation of mass throughout the experiment.

4.2.4 Measurements and Quantification.

Bromide conservative tracer and Bromide ions: Bromide (ion) concentrations were determined using a HI4102 Bromide Combination Ion Selective Electrode in conjunction with a Benchtop pH/ISE/mV/Temp meter (model HI3222-02). This electrode was calibrated using 0.1 M Bromide Standard, HI 4002-01. A 799 ppm bromide standard was created by diluting 100ml HI4002-01 0.1M Bromide standard solution into 1 litre deionised water. Using volumetric pipettes and glassware, this was then serial diluted to bracket the sample concentration. Solutions below 80ppm were prepared fresh for use, while solutions of higher concentration were stored in dark sealed bottles and kept out of the light.

Silver nitrate: Silver (nitrate) concentrations were determined using a HI 4115 silver/sulphide combination ISE electrode, again used with the Benchtop pH/ISE/mV/Temp meter. This electrode was calibrated using 0.1M Silver standard solution, HI-4015-01. A 1078.7 ppm silver standard was created by diluting 100ml of HI 4015-01 0.1M Silver standard solution into 1 litre deionised water, then using volumetric pipettes and glassware, this was then serial diluted to bracket the sample concentration. Solutions below 100ppm were prepared fresh for use, while solutions of higher concentration were stored in dark sealed plastic bottles and kept out of the light.

Silver Nanoparticles: The silver/sulphide combination ISE electrode was not receptive to AgNPs concentrations. Instead the photometer protocol as described

in Chapter 3 was used for all AgNPs samples resulting from the transport run. To ensure the continued accuracy of these results 8 or more samples per run were sent to the Analytical Research Facility (ARF), Plymouth University for ICP-MS and ICP-OES analysis and validation (again method described in Chapter 3). Prior to transport to Plymouth University, the samples were sonicated for 20 mins and then securely contained within clean 15ml plastic vials and wrapped in foil so as to protect the sample from UV light interference.

Bulk Silver: Once again the silver/sulphide combination ISE electrode was not receptive to bulk silver concentrations. Nor was the photometer protocol suitable for determining the bulk silver concentrations. Selected samples were instead sent to the Analytical Research Facility (ARF), Plymouth University for ICP-MS and ICP-OES analysis. Again, prior to transport to Plymouth University, the samples were sonicated for 20 mins and then securely contained within clean 15ml plastic vials, wrapped in foil.

4.3 Results and Discussion

4.3.1 Column experiments with quartz sand

For each contaminant column run, concentration results were first flow corrected in accordance with the conservative tracer results. The results were then normalised and mass balances were calculated in accordance with the law of conservation of mass states in an isolated system.

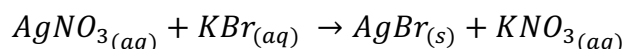
$$\text{Total mass entering system} = \text{total mass leaving the system}$$

Actual influent concentrations (Table 4.2) were noticed to vary slightly from theoretical calculations. Specifically, in the case of AgNPs and Bulk silver, influents were made up immediately for use from stock supplies. As such actual concentrations were not confirmed until well after the run and sample results had been returned from Analytical Research Facility (ARF), Plymouth University.

	Flow rate (ml/min)	Pulse volume (ml)	Conc. (ppm)	Pulse Conc. (ppm)	Total Mass Balance (ppm)	Recovery (%)
Conservative tracer	7.57	300	115	34.5	34.47	100
AgNO3 C1	7.57	300	102	30.6	0.002	0
AgNO3 C2	7.57	300	102	30.6	0.002	0
Conservative tracer	7.47	300	127	38.1	35.76	94
AgNPs C1	7.47	300	23.69	7.11	6.74	95
AgNPs C2	7.47	300	23.67	7.101	6.47	91
Conservative tracer	7.23	300	111	33.3	31.05	93
AgNPs C3	7.23	300	14.9	4.47	4.409	99
AgNPs C4	7.23	300	12.9	3.87	3.85	100
Conservative tracer	7.09	300	145	43.5	42.66	98
Bulk silver C1	7.09	300	20.56	6.17	1.12	73
Bulk silver C2	7.09	300	20.56	6.17	1.04	67

TABLE 4.4: Table detailing the key column parameters for AgNO₃, AgNPs and Bulk Silver transport runs through columns packed with quartz sand.

The mobility of the three silver species across the quartz sand bed differs considerably (Figure 4.5). 100 per cent retention of the AgNO_3 within the media bed was recorded, circa 30 per cent of the bulk, while in contrast there was minimal to zero retention of AgNPs. The retention of AgNO_3 was further confirmed by the behaviour of the bromide indicator, which was run directly after each contaminant column run. For the AgNO_3 columns, between 34 and 35% of the bromide was retained, mostly likely in the form of the halide silver bromide, an insoluble salt, following the below reaction.



It is likely that other chemistry's such as oxidisation and the precipitation of other silver salts may have occurred within the sand column, though these were not tested for. In contrast, 92 – 95% mass recovery of the bromide indicator was achieved following the AgNPs and bulk silver column runs, which in the case of the AgNPs provides some supporting confirmation that there was minimal to no (reactive) silver remaining in the system available for the above halide reaction; while in the case of bulk silver, such a halide reaction would not be expected due to the reduced reactive availability of the bulk parent material. All effluent samples were measured for temperature and pH (Figure 4.6 and Figure 4.7). No significant variation in either parameter was observed during any of the quartz sand transport runs. For AgNO_3 the temperature of all effluents maintained at an even average of 24.53°C (Stdev 0.1463) and a gently fluctuating pH at an average of 6.9 (Stdev 0.2921). For AgNP effluents, the temperature of all effluents samples observed an

average of 15.57°C (Stdev 0.1401) and an average pH of 7.13 (Stdev 0.5167). For [bulk] silver effluent samples, the average temperature was recorded at 17.52°C (Stdev 0.1228) and with an average pH of 7.4 (Stdev 0.2299).

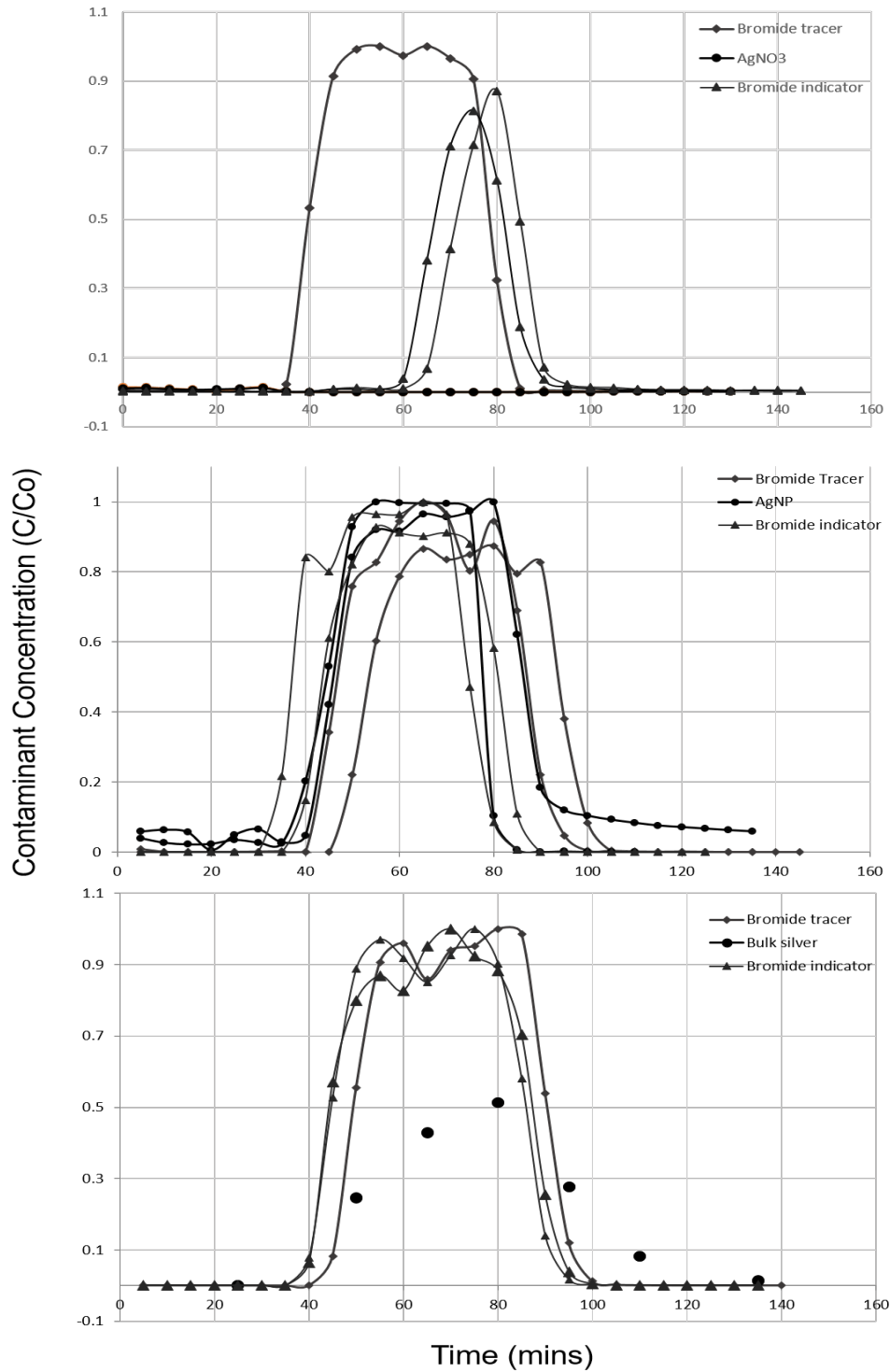


FIGURE 4.5: Three plots displaying the mobility of three speciations of silver (Silver nitrate, Silver nanoparticles, Bulk silver) across acrylic columns, 6cm in diameter and 30cm in length, wet packed with quartz sand. All results expressed as mean

values in mg/L over time (mins). Top, Silver nitrate (AgNO_3). Middle Silver Nanoparticles (AgNPs). Bottom, Bulk Silver (Ag).

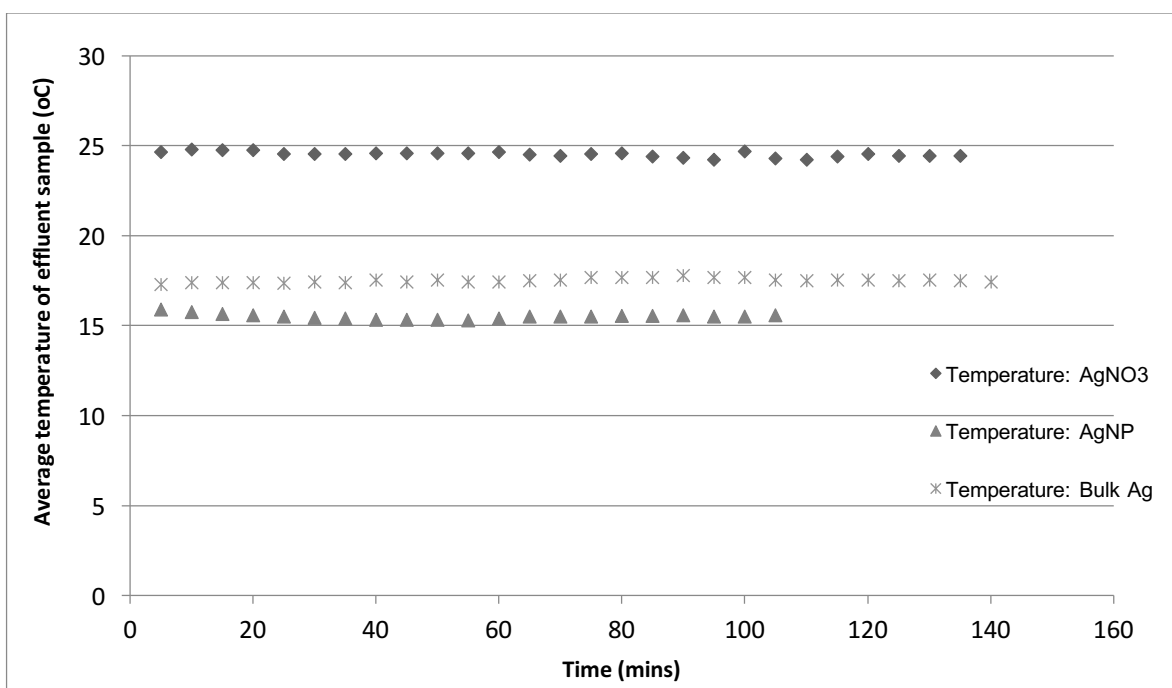


FIGURE 4.6: Measured Temperature ($^{\circ}\text{C}$) values of column effluent samples, expressed as mean values ($^{\circ}\text{C}$) over time (mins). Standard deviation of AgNO_3 Temperature values 0.1463°C ; Standard deviation of AgNPs Temperature values 0.1401°C ; Standard deviation of Bulk Ag Temperature values 0.118°C .

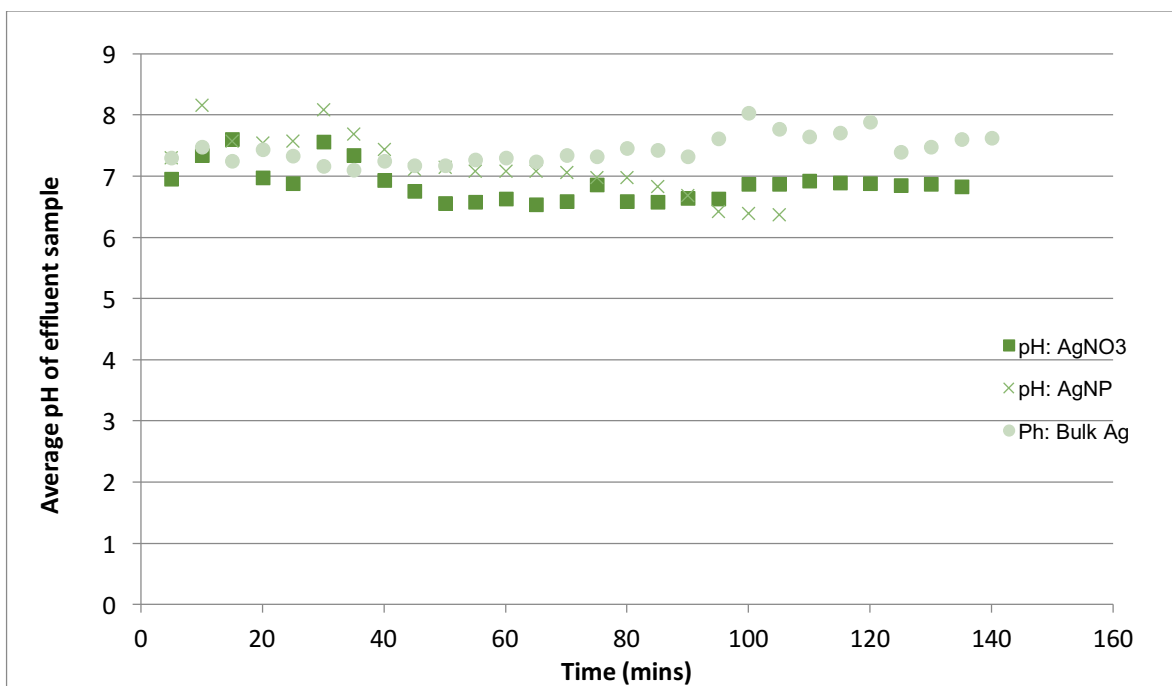


FIGURE 4.7: Measured pH values of column effluent samples, expressed as mean values (pH) over time (mins). Standard deviation of AgNO_3 pH values 0.2921; Standard deviation of AgNPs pH values 0.5167; Standard deviation of Bulk Ag pH values 0.2299.

The breakthrough curves obtained from the three species of silver (AgNPs, AgNO₃ and bulk) showed that the nano-silver effluent of nominal concentration 20ppm reached a plateau after four pore volumes (Figure 4.10). The slightly lower AgNPs concentration of 10ppm didn't quite reach complete breakthrough, with 91 and 95 percent mass recovery being recorded for these runs, in contrast to 99 and 100 per cent for the 20ppm AgNPs. Bulk silver demonstrated delayed initial breakthrough, after circa 3 pore volumes in comparison to the AgNPs, but displays a lower, more flatten profile in line with circa 30 per cent media bed retention. No breakthrough was observed for the AgNO₃.

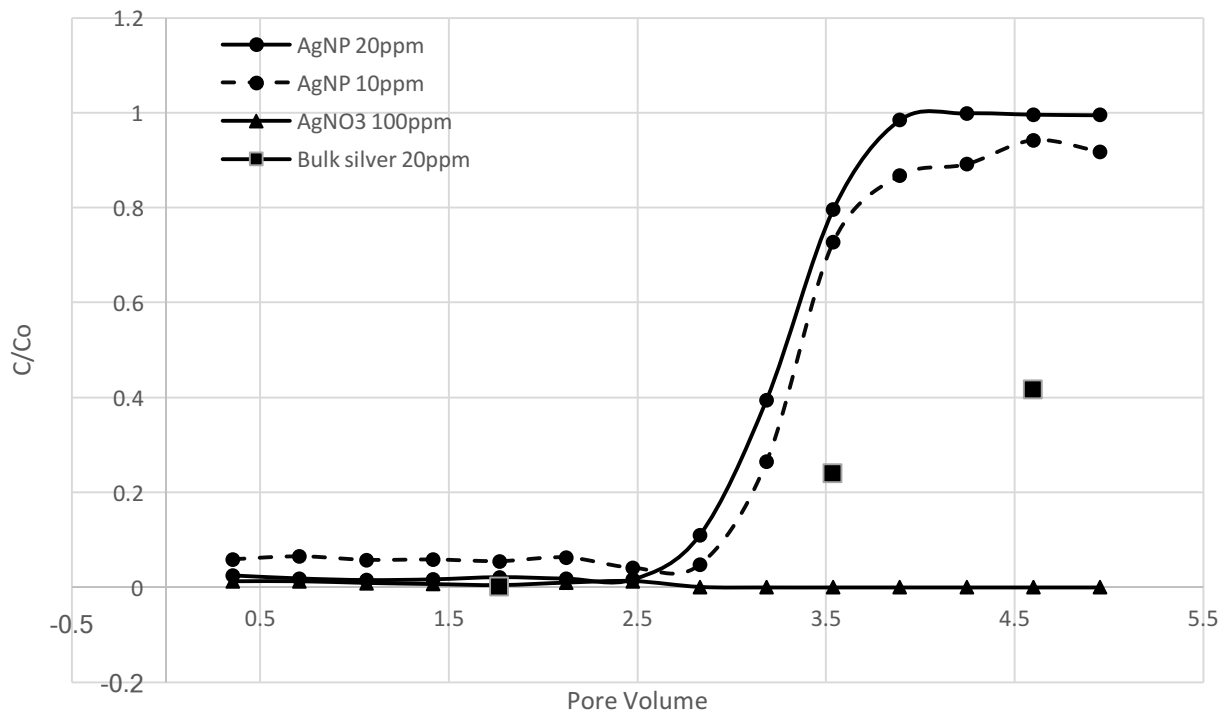


FIGURE 4.8: Breakthrough curves of three silver species AgNPs (at nominal concentrations of 10 and 20ppm), AgNO₃ (100ppm) and bulk silver (10ppm), showing the change in normalized effluent concentration (C/C_0) as a function pore volume.

AgNO_3 with solubility in water of 256 g/100 mL (25 °C) appears to have been the most available silver species for surface adsorption and deposition to the fixed media bed and / or reaction with ligands present, forming silver oxides and halides, and as such becoming trapped within the media bed in solid phases such as insoluble salts.

The mobility of the AgNPs through the quartz sand columns was contrary to the prior expectation that van der Waals forces would have acted to trap some if not all of the AgNPs. This can be attributed to two factors. Firstly, that the reduction agent used as part of the synthesis (sodium borohydride, NaBH_4) is likely to have contributed a negative surface charge to the AgNPs. While sodium borohydride was not used as a surface capping agent, it is likely that contamination from the hydrogen atoms in the BH_4^- anion, which aid in the chemical reduction process of AgNPs, also resulted in surface contamination with borides (Bönnemann et al. 1994; Sardar et al. 2007; Tolaymat et al. 2010). Thus forces of repulsion would dominate between the AgNPs and the negatively charged silica surfaces of the quartz sand bed; where silica, when immersed in water is often known to acquire a negative surface charge density, primarily through the dissociation of terminal silanol groups (Behrens & Grier 2008; Tolaymat et al. 2010). It is also observed for nanoparticles, that as particle size decreases, so do van der Waals forces; so that electrostatic interactions at the nanoscale are more likely to have the greater influence over surface interactions in aqueous media (Darlington et al. 2009). Secondly, it is also possible that the presence of Al_2O_3 (and Au, see chapter 5) within the sand composition, contributed to a low level of surface passivation of the SiO_2 and AgNPs surfaces by providing a surface layer to the silica in the aqueous, hindering any further reaction or interaction with the chemistry of its' environment

(Dingemans & Kessels 2012). This theory of surface passivation is supported by the mobility of bulk silver through the quartz sand media, where attraction forces such as van der Waals would have been expected to be more effective in capturing more of the bulk silver particles of positive surface charge. It can also be noted, that the small size of the AgNPs (~10nm diameter) will have ensured little to no resistance or friction for the effective mobility of the AgNPs through the sand column pore spaces.

It was observed that the bulk silver travelled more slowly through the quartz sand columns, making its' initial break through 5 to 10 minutes after the bromide / silver nitrate / AgNPs influent samples and taking up to a further hour after the other contaminants to completely pass through. This is likely the result of increased particle size, resulting in forces of friction and collision interference within the media bed. The bulk silver was also notably insoluble, requiring considerable agitation and sonication during the influent feeding process to keep the particles in suspension. Inside the sand column, it is likely these insoluble particles would have more readily settled out into the available pore spaces, with only the incoming flow as encouragement to move further up (against gravity) and out of the system.

4.3.2 GAC Isotherms

A column transport trial using the same method as for the quartz sand media was conducted for GAC using the conservative bromide tracer. The trial was run for 7.4 hours towards the end of which maximum concentrations of bromide were just starting to breakthrough. Initial bromide breakthrough occurred at 150 minutes. A simple projection based upon the first 7.4 hours of results suggested that a carbon

column run to 100% mass recovery would have taken 96.9 hours (Figure 4.9).

Hence the motivation to favor GAC Isotherm batch reactors.

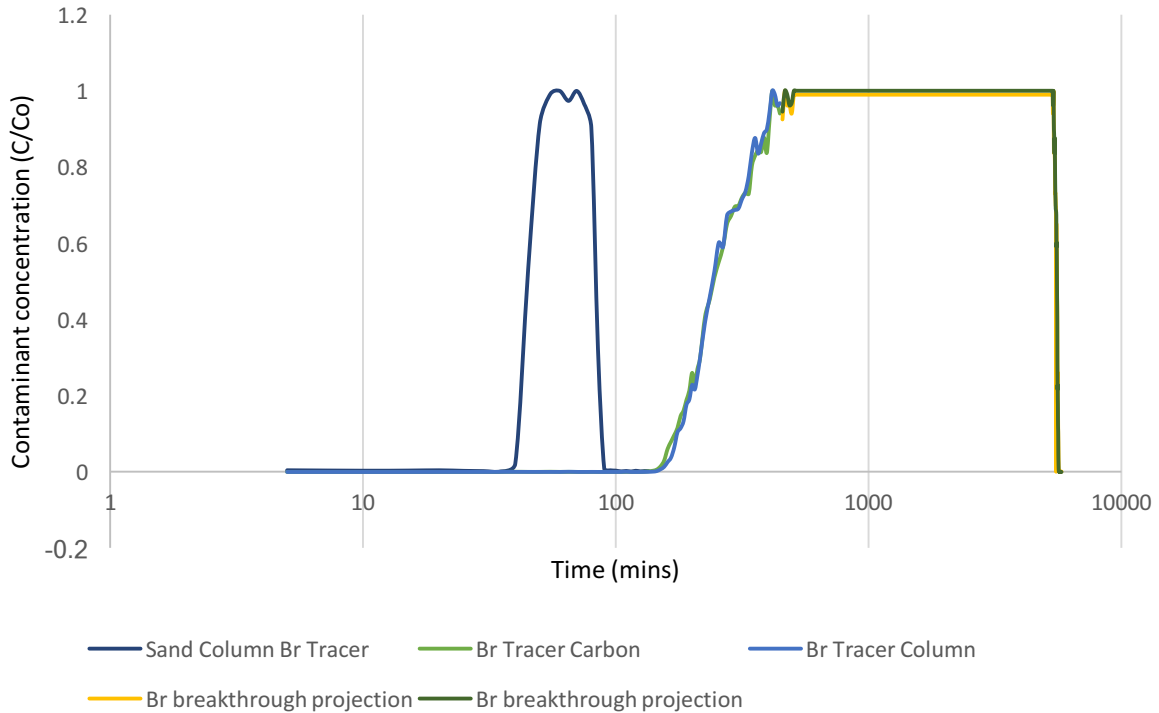


FIGURE 4.9: Breakthrough curve, including an estimated projection for the transport of the bromide tracer across two columns of GAC. Graph includes bromide tracer breakthrough curve for sand for comparative purposes.

GAC adsorption kinetics showed, as expected good adsorption efficiencies for both AgNO_3 and also AgNPs but not for bulk silver. AgNO_3 was the most readily adsorbed by the GAC, with full contaminant absorbance being achieved at 5 grams of carbon dosage. AgNPs adsorption behaved in a similar fashion being fully adsorbed at 12 grams of carbon dosage (Figure 4.8).

The adsorption batch reactor tests showed insufficient evidence that the bulk silver was adsorbed by the GAC. This was contrary to expectation where typically GAC is known to effectively removed contaminants within a range of 0.5 – 50 micrometers in particle size; where the bulk silver particles were of 300 – 800nm or 0.3 – 0.8 μ m in particle size, just within the lower range of absorbance. This particular result may be attributed to both the high pH levels of the supernatant (GAC pH aqueous phase of 10), where Jia and Demopoulos (2003) observed that low pH and high salt concentrations were detrimental to the adsorption of bulk Ag on activated carbon (Jia & Demopoulos 2003), but also to the insolubility of the bulk silver and its propensity to settle out from a state of suspension (despite encouragement via an action of repeated sonication). In general, complications were encountered during the mixing and latterly the final sampling of each batch reactor. Even with sonication, it was challenging to ensure that the samples collected for analysis were representative of the whole. ICP-MS and ICP-OES returned results (Table 4.3) for the bulk silver samples that showed little to no adsorption.

10 minutes after the addition of the GAC to the batch reactors, the supernatant was tested for temperature and pH as well as sampled for AgNO₃, AgNP and Ag. (Figure 4.11 and Figure 4.12). No significant variation in either parameter was observed across any of the AgNO₃, AgNP or Ag batch reactors, where for AgNO₃ sampling points temperature maintained an average around 19.62 $^{\circ}$ C (Stdev 0.0.2939). For AgNP the average temperature was recorded at 18.95 $^{\circ}$ C (Stdev 0.0743), and for Ag supernatant samples, an average temperature of 18.2 $^{\circ}$ C (Stdev 0.2)

With regards to pH, this was noted to fluctuate to some degree, rising up to around 8.32. For AgNO₃ the average pH was recorded at 7.2 (Stdev 1.2050). For AgNP, an average of 7.03 (Stdev 1.3648) and for bulk Ag an average pH of 7.00 (Stdev 1.297). This slight rising in pH is largely to be expected due to the influence of the activated carbon with in solution

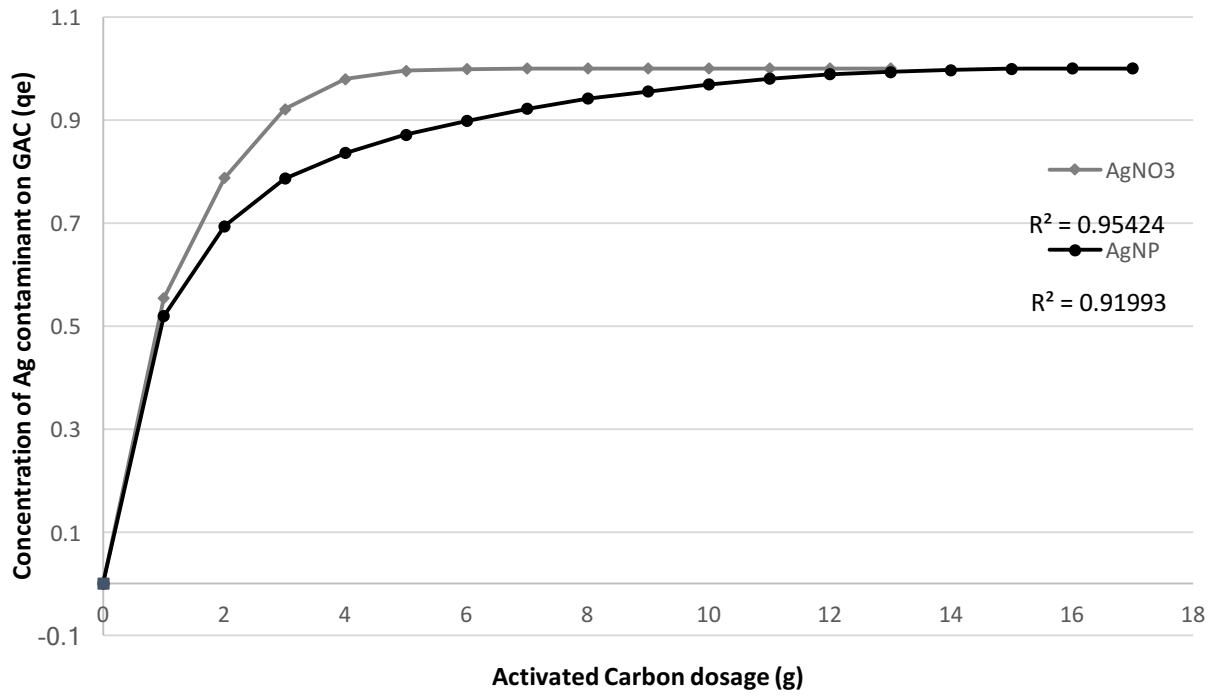


FIGURE 4.10: Adsorption kinetics for silver speciations nitrate and nanoparticles.

Third order polynomial fit: AgNO₃ R² 0.9542; AgNPs R² 0.9199

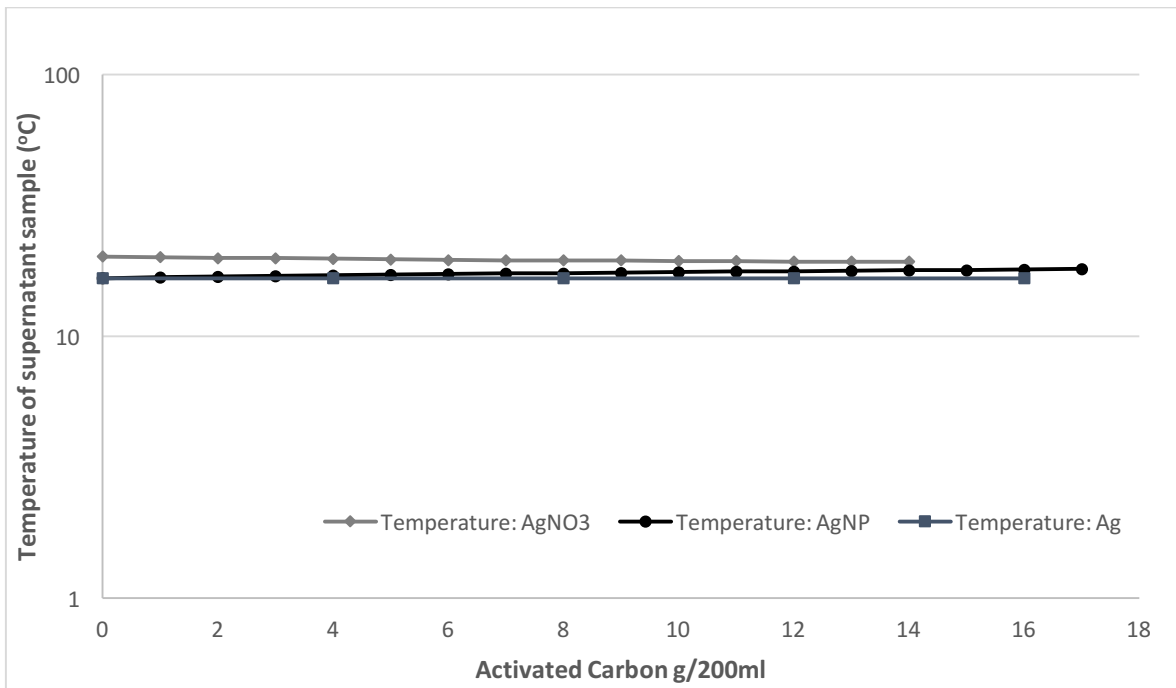


FIGURE 4.11: Recorded temperature (°C) of the GAC Isotherm Supernatant, expressed as grams of activated carbon per 200ml against determined values of temperature (°C). Standard deviation of AgNO₃ values 0.0.2939; Standard deviation of AgNPs values 0.0743; Standard deviation of Bulk Ag temperature values 0.2.

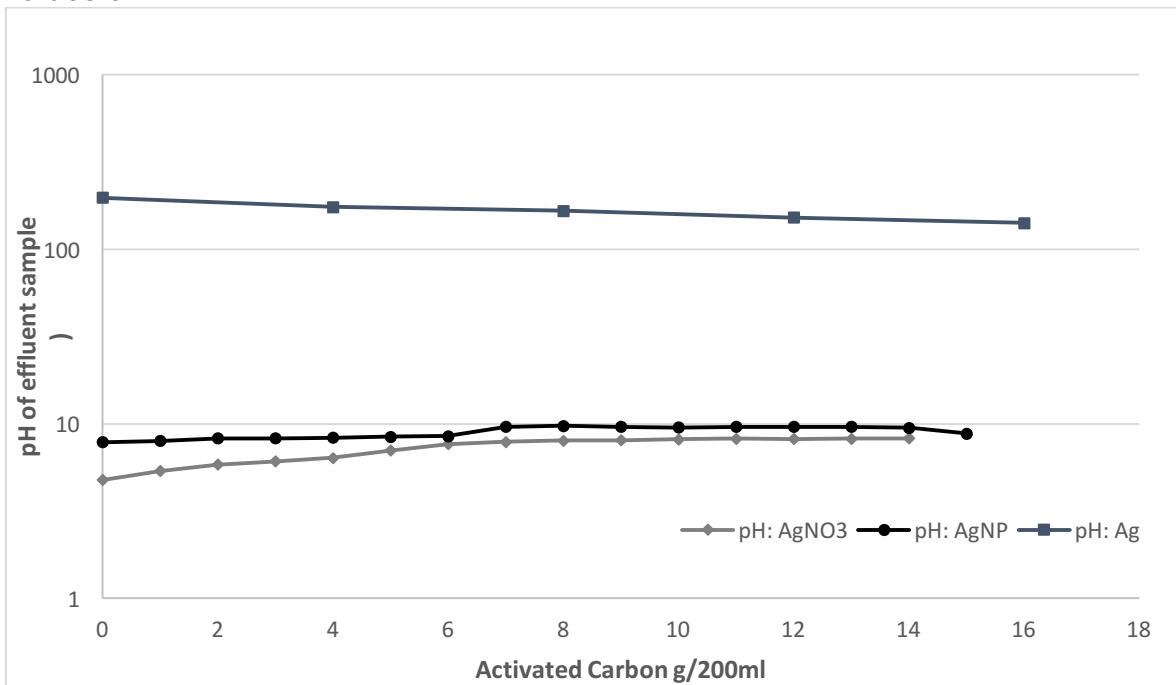


FIGURE 4.12: Measured pH of the GAC Isotherm Supernatant, expressed as grams of activated carbon per 200ml against determined values of pH. Standard deviation of AgNO₃ pH values 1.2051; Standard deviation of AgNPs pH values 1.3648; Standard deviation of Bulk Ag pH values 0.1.2966.

GAC (g)	Co (mg/L)	Ag (mg/L)	Ag (mg/L)	pH	Temp
0	10	13.06	13.26	5.42	18.9
2	10	12.42	12.60	8.53	18.8
6	10	18.18	18.52	9	18.8
10	10	16.09	16.30	9.17	18.9
14	10	18.47	18.81	9.24	19.1

TABLE 4.5: Isotherm batch reactor results for bulk silver

For the GAC isotherms C_e was measured as part of the experimental procedure and q_e calculated as

$$q_e = \frac{x}{m}$$

Where x is the mass of adsorbate and m is the mass of adsorbent. Expressed as units.

$$q_e = \frac{mg}{g}$$

Adsorption isotherms (Figure 4.13) show a good power fit with the Freundlich Isotherm model. AgNO_3 being efficiently adsorbed on to the carbon, while the AgNPs adsorption behaviour was slightly more erratic yet still achieved a fit of R^2 0.8566. This may be explained by the fact that the Langmuir isotherm assumes monolayer coverage on a homogeneous surface with equal adsorption sites. This even distribution is easier to achieve with a solute in full suspension such as the AgNO_3 . In contrast, the AgNPs while well dispersed in solution, and subject to ample mixing, may have not achieved a full even distribution across the GAC surface due to colloidal aggregation. Following the creation of the isotherm graph (Figure 4.13) it was possible to determine the Freundlich adsorption constants for AgNO_3 and AgNPs (Table 4.4).

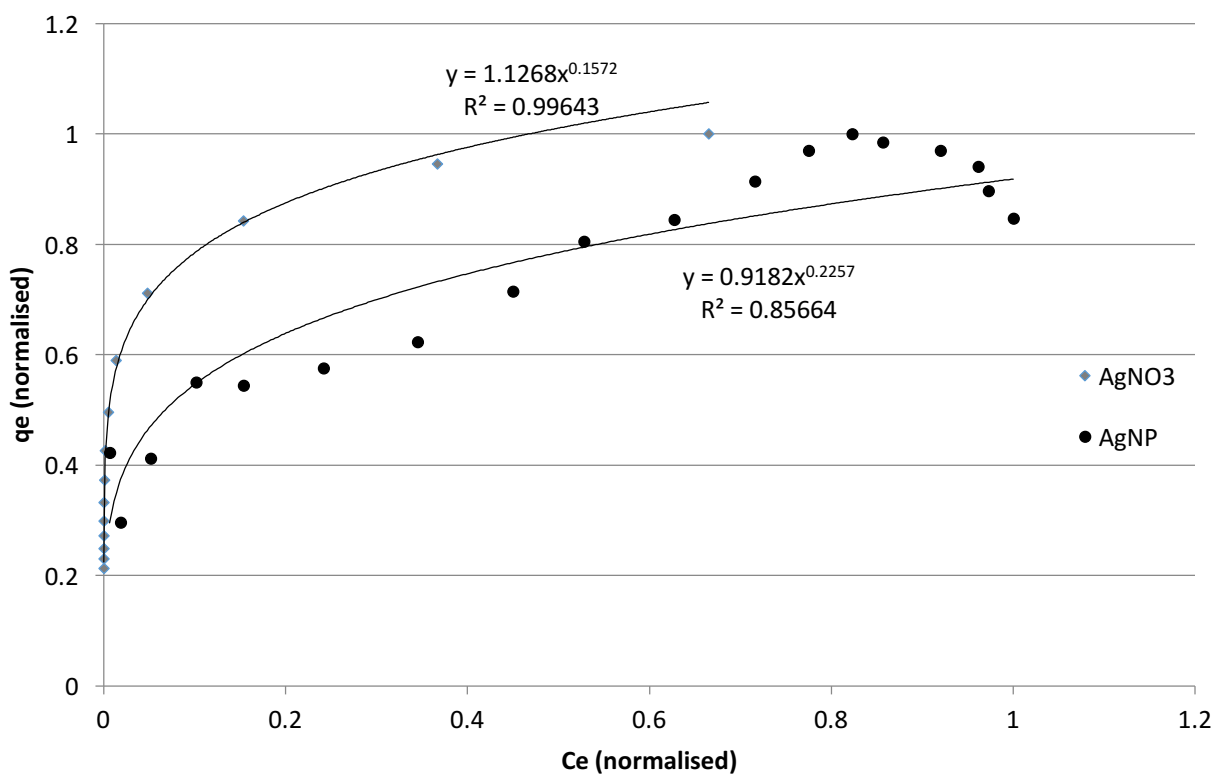


FIGURE 4.13: Adsorption isotherm. Amount of solute adsorbed (q_e) plotted against equilibrium concentration of solute remaining in solution. All data normalised.

Determination of Freundlich adsorption isotherm constants		
	AgNO ₃	AgNPs
K	0.2134	0.2962
1/n	1.12368x0.1572	0.9182x0.2257

Table 4.6: AgNO₃ and AgNPs Freundlich constants.

4.4 Limitations

- This experimental set-up simulates artificial quartz sand and activated carbon filtration techniques and have employed simple influents consisting of a solute dissolved or suspended in a solvent, in this case water. As result, this experiment and resulting data cannot be considered representative of real-world systems. Its advantage however is that it provides a simplified study that investigates the transport / sorption of AgNO₃, AgNPs and bulk silver (Ag) through / to quartz sand and activated carbon (respectively) in an uncomplex manner, that is easily comparable and replicated. This study can be used to form the foundation of more complex studies that seek to gradually factor in various particle specific and environmental factors, such as synthetic wastewater complexes, variations in influent solute concentration, to include both pristine influent presentations and “age” effluents recycled as influents. .
- The size of the arclitic columns used for quartz sand were not compatible for the running of laboratory columns for GAC. This was due to the experimental run time that was required to (via continuous running) achieve contaminant break-through. As such the resulting data for quartz sand and GAC is not directly comparable. However, the GAC isotherms employed are representative of more typical / common laboratory methods for the sorption analysis of GAC.
- As a result of the quantitative measuring instrumentation available, certain variables were restricted to the measurement ranges of the instrumentation. It would have been preferable and closer to real-world AgNP concentration values to have lowered the maximum influent solute concentration to 0.1mg/L or less. This however, gave way to unreliable results from both the Silver/sulfide ISE electrode and Palintest Photometer. As these were the only *in-situ* methods

of determining Ag^+ and AgNO_3 concentration, they became a governing factor for all influent concentrations chosen.

- Due to lack of access to the appropriate equipment and instrumentation, this study did not record important nanoparticle characteristics such as zeta-potential or dissolution rates, the latter particularly represents highly valuable and informative piece of data describing the stability and solubility of the AgNP and other silver species measured.
- The handling, employment and quantitative measurement of bulk silver (Ag) samples presented several challenges that reduced the integrity of the resulting data. Firstly, the silver/sulphide combination ISE electrode was not receptive to bulk silver concentrations, nor was the photometer protocol suitable. As a result, far fewer samples for both the quartz sand columns and GAC isotherms were sampled for Ag concentration (mg/L). Secondly, due to their large particle size / mass, they exhibited a propensity to repeatedly settle out of suspension. To ensure consistency in the Ag influent feed for both the quartz sand and GAC experiments, the influent samples were regularly subjected to sonication and continuous subjected to orbital mixing.
- The silver/sulphide combination ISE electrode was not receptive to AgNPs concentrations. Instead the Palintest Photometer was used to determine AgNPs concentrations (mg/L) in all quartz sand column and GAC [effluent] samples. The protocol for which has been described in Chapter 3. To ensure the integrity of these result however, >8 samples per run were sent to the Analytical Research Facility (ARF), Plymouth University for ICP-MS and ICP-OES analysis and validation.

4.5 Conclusion

The aim of this study was to investigate the transport of AgNPs in comparison to Silver nitrate and bulk silver through media beds such as quartz sand and GAC. Column studies were employed for the quartz sand and isotherm adsorption batch studies for the GAC. Conclusions from the present study are as follows.

- i. AgNPs were highly dynamic through the quartz sand media columns. This is credited to the negative surface charge of both the silica surface of quartz sand and also the AgNPs, resulting in forces of repulsion that acted to propel the AgNPs through the system. Uncoated AgNPs would ordinarily be expected to exhibit a positive charge, however in the case of the laboratory synthesized AgNPs used in this study, a negative surface charge was acquired as a result of surface contamination from boride ions, donated by the reducing agent sodium borohydride. This highlights the importance of considering the surface properties of AgNPs, as a result of a) the method of synthesis used and b) the choice of capping / stabilizing agent applied, as they can be expected to have a significant influence upon the mobility of AgNPs through a variety of urban and natural environments.
- ii. The high mobility of the AgNPs can also be attributed to their small particulate size of average 9.98nm diameter, which would have ensured their unimpeded passage through the flooded pore spaces of the sand column. The insoluble bulk silver comparison, at particle sizes ranging between 300 – 800nm, demonstrated a reduced mobility through the quartz sand media, where van der Waals forces of attraction and other forces of friction, collision, and gravity would have been greater and together acted to encumber the transport of these larger particles through the system. As

such, AgNPs size is an important factor influencing mobility, where pristine AgNPs present in an un-aggregated or agglomerated form are more likely to be discharged from wastewater treatment systems into the receiving environment. This presents a reasonable level of concern for aquatic life in the receiving environment, where toxicity is considered to be AgNPs size correlated; increasing in lethal potential with the decrease in AgNPs size.

- iii. In contrast, AgNO_3 was not observed to be mobile through the quartz sand column. Due to its solubility, it appears to have been more readily available for surface adsorption, oxidation and chemical reaction with ligand residues present.
- iv. Bulk silver was noted to be partially mobile through the quartz sand column, achieving circa 70% mass recovery in the column effluent. However, it was observed to travel at a slower rate of progression; most likely attributable to its larger particle size of 300 – 800nm (diameter), and as such subject to greater forces of friction and collisions within the quartz sand media environment. It had been anticipated that more bulk silver would have been captured by the quartz sand media column, though it is considered possible that the presence of Al_2O_3 within the quartz sand composition may have led to some low level surface passivation of the silica surfaces.
- v. There was a good degree of adsorption of the AgNO_3 and AgNPs onto the GAC media. This was confirmed with the Freundlich adsorption isotherm, where AgNO_3 gave a good fit of R^2 0.9964 to the Freundlich isotherm theory, while AgNPs less so at R^2 0.8566. The effective adsorption of AgNO_3 can be attributed to its' solubility within the supernatant, thereby achieving an even coverage of the GAC active surface, thus increasing the

opportunity for adsorption. In contrast for AgNPs, a slower adsorption rate was observed. While AgNPs are not soluble, they exhibit a high degree of suspension in solution, which would have ensured good coverage of the GAC but not equal to that of AgNO₃ via processes of colloidal aggregation. This reinforces the requirement to understand more completely the zeta-potentials (an indicator of stability of colloidal dispersions) of AgNPs under varying influences of size, surface coatings and following processes and exposures that result in particle aging and transformation.

- vi. This particular study observed that GAC was not effective at adsorbing bulk silver of particle sizes 300 – 800nm. Considerable challenges were experienced with the handling of this product due to its' insolubility and inherent propensity to quickly settle out of a forced state of suspension. As a result, despite efforts of continued agitation and sonication it is not likely that this contaminant would have achieve a suitable level of coverage and contact time with the surface of GAC for adsorption to take place. It is also noted that previous studies have found that the adsorption of bulk silver to GAC to be a low pH and high salt concentration dependent process, for which in this study, such conditions were not achieved.

CHAPTER 5

PARTICLE SIZE DISTRIBUTION OF AgNPs LABORATORY COLUMN AND BATCH REACTOR

EFFLUENTS

5.1 Introduction

Nanomaterials are typically characterised by materials of which any one unit is sized, in at least one dimension, between 1 and 100nm. Since the development of the Scanning Tunnelling Microscope in 1982, there has been a surge of interest in the wide range of new and unique properties that bulk materials exhibit at the nanoscale (Dowling et al. 2004). Nanoparticles variants of bulk metal materials have received particular interest for their unique mechanical, electrical, magnetic, optical and chemical properties, among many other properties that could be mentioned. More recently, a range of nanoparticles, including that of silver nanoparticles (AgNPs), have become the focal point of research for their potential in biomedical applications, such as tissue engineering, drug delivery and biosensors. In addition to the above, existing within the size range of 1 – 100nm the material study of nanoparticles is helping to bridge the gap of understanding between bulk parent materials and that of their associated molecular and atomic structures. Where the bulk parent material can typically be expected to exhibit a predictable range of characteristics and properties regardless of size / mass, its' nano variant may exhibit a wide range of size-dependant variables and properties.

5.1.1 Why size matters

Over the last 20 years' materials researchers have been comprehensively working toward understanding, enhancing and harnessing the size-dependant properties of AgNPs across a wide range of applications spanning industry, medicine, domestic and personal hygiene sectors (Peretyazhko et al. 2014; Xiu et al. 2011). For which, the literature suggests that AgNPs, spherical and with a diameter of less than

20nm are the most common found across the majority of commercial applications such as paints, textiles and clothing, cosmetics, and prophylactics (Benn & Westerhoff 2008; Rezić 2012; Kaegi et al. 2010; Pasricha et al. 2012; Blaser et al. 2008). The most common applications of AgNPs include applications that take advantage of their antibacterial properties, where AgNPs 10nm or less, typically exhibit greater toxicity than AgNPs of larger dimensions (Hussain et al. 2009; Liang et al. 2010; Morones et al. 2005). Much of this is attributed to the resulting increase in surface area to volume ratio, which in turn promotes increased surface reactivity and surface ion release, which is known to be particularly toxic to both micro and other aquatic organisms (Cronholm et al. 2013). Where particles sizes range between 1 – 10nm their ability to attach to cell membranes and cause disruption to membrane function is amplified, or indeed their ability to completely penetrate the cell entirely, causing (often terminal) internal damage by interacting with intracellular proteins (Morones et al. 2005). However, these advantageous anti-bacterial, anti-microbial properties outside of the context of medicine and personal health care regimes can take on a far less desirable appeal. Within the context of wastewater treatment plants and the natural environment, the anti-microbial properties of AgNPs are a cause for concern, where there exist communities of microbes essential to the health and wellbeing of the overall WwTP or natural (eco)system. In this case, a more complete knowledge AgNPs size dependant mobility and toxicity can provide a valuable indicator of AgNPs fates.

5.1.2 Synthesis methods to control size

Silver nanoparticles can be synthesized by several methods; differing techniques being favoured for the resulting shapes, sizes and structures that they favour. The most common technique is that that falls into the category of wet chemistry, of which several methods exist for AgNPs, though they are all based upon the same principal of a silver complex, typically a salt, being reduced to a state of colloidal silver (Natsuki et al. 2015). Once formed the AgNPs can also be stabilised using a capping agent; a chemical additive designed to prevent particle aggregation or agglomeration. The choice of solvents, reducing and capping agents used as part of the reduction process can all exact slightly different outcomes with regards to the physical and morphological characteristics of the resulting AgNPs. In turn, these specific characteristics will influence the fate, transport and toxicity of the AgNPs through various urban and environmental compartments. For example, silver nitrate reduced with sodium borohydride and / or capped with sodium citrate will form a AgNPs with a negative surface charge, which therefore can be expected to behave differently to one with a positive surface charge (Peretyazhko et al. 2014; Tolaymat et al. 2010).

5.1.3 Problems associated with size

Smaller particles, manufactured with capping agents to ensure colloidal stability, may prove more persistent and mobile through a range of wastewater treatment filtering processes, thereby ensuring their presence in the final effluent discharge and subsequently the aquatic environment (Zhang & Zhang 2014). Negatively charged AgNPs moving through a negatively charged sand or soil column will be

propelled forward as a result of repulsion forces (Tolaymat et al. 2010). While those capped with branched polyethyleneimine (BPEI) as one example, would result in a positive surface charge and as such would be more likely to become trapped within the same column due to attraction (van der Waals) forces. Surface charge will also impact upon reactivity rates of the AgNPs with available ligands, where again the presence of the negative surface charge may hinder common silver reactions and chemical associations with a range of negatively charged salts such as chloride and sulphide commonly found in wastewater streams.

5.1.4 Aims and Objectives

This chapter presents a comparative AgNPs influent and effluent particle size distribution (PSD) analysis, seeking to understand the physical and chemical effects that quartz sand and granulated activated carbon (GAC) filtration medias have on AgNPs effluents. The AgNPs samples employed in this study were synthesised as described in Chapter 3 and the effluents all originated from the transport and adsorption isotherm studies presented in Chapter 4. AgNPs particle size can be defined by the initial size they were synthesised / manufactured to, but also as a result of the physical and chemical influences of aggregation, agglomeration and / or a change in chemical speciation resulting from the environments that they have been exposed to. A better understanding of AgNPs transformation and subsequent speciation under relevant wastewater treatment conditions, such as tertiary filtration processes, will allow for a more informed understanding of their resulting fate following discharge into the aquatic environment. Work packages in this section include:

- a) The preparation of influent and effluent samples for Transmission electron microscopy (TEM) and Energy-dispersive X-ray spectroscopy (EDS) analysis,
- b) Particle size distribution (PSD) analysis of AgNPs TEM images, looking for normal distribution patterns in the influent samples, and for evidence of skew and kurtosis in effluent samples, thus indicating a change in particle size of AgNPs following exposure to media environments,
- c) Elemental analysis of the influent and samples utilizing EDS results, looking for evidence of any change in the chemical composition of the AgNPs.

5.2 Methods and materials.

5.2.1 TEM and EDS

Samples were prepared on copper mesh 400 holey carbon film (Agar Scientific, Electron Technology UK Ltd). 1 drop measured from a microliter pipette (Fisherbrand) was dropped onto the grid and left to dry out thoroughly for 2 days prior to transmission electron microscopy (TEM) analysis. Images were obtained using a 200 kV TEM (JEM-2100 LaB6). Energy-dispersive X-ray spectroscopy (EDS) analysis was performed to provide an assessment of the elemental composition of the sample, where the presence of copper (Cu) and carbon (C) can be attributed to the sample presentation grid.

5.2.2 Particle measurements

Diameter measurements were acquired from the TEM images using the GNU Image Manipulation Program (GIMP). Using the measuring tool provided in the software, first the scale bar for each TEM image was measured and converted into pixels. Particle diameters were then measured in pixels and later converted back into nanometres for analysis. For the purposes of data standardisation, particles were measured under the assumption that all particles were sphere shaped (Horiba Instruments INC. 2010).

5.3 Results and Discussion

5.3.1 TEM imaging of AgNPs influents and effluents.

AgNPs Influent TEM imaging confirms the homogeneous dispersion of AgNPs particle size and shape formed, with an average size of individual particles found to be 9.98nm in diameter, with a standard deviation of 3.37nm, 3.43nm and 2.54nm respectively for AgNPs Influent (a), (b) and (c) (Figure 5.1). Confirmation of their silver composition can be seen in both the unique d-spacing (regularly spaced electrons that can be seen in shaded planes) visible in several of the TEM images, as well by the accompany EDS analysis, where there is a clear silver (Ag) peak against a backdrop of carbon (C) and copper (Cu) arising from the holey carbon copper grid that the sample is presented on (Figure 5.3 (a)).

In contrast the AgNPs effluent samples, were considerably more heterogeneous in nature due to both particle agglomeration and aggregation. Quartz sand effluent samples particularly display visual evidence for particle heterogeneity, including indications of surface coatings acquired and a tendency to aggregate and agglomerate. Figure 5.1 Quartz sand effluent (b) is a particularly striking example. Of the carbon effluent samples, first of all it is apparent that concentration of AgNPs on the grid was less than that of its sand effluent and influent comparisons'. An increase in heterogeneity with the sample is visually apparent as is the tendency for particle aggregation, however not to the same extent as that of the sand effluent samples.

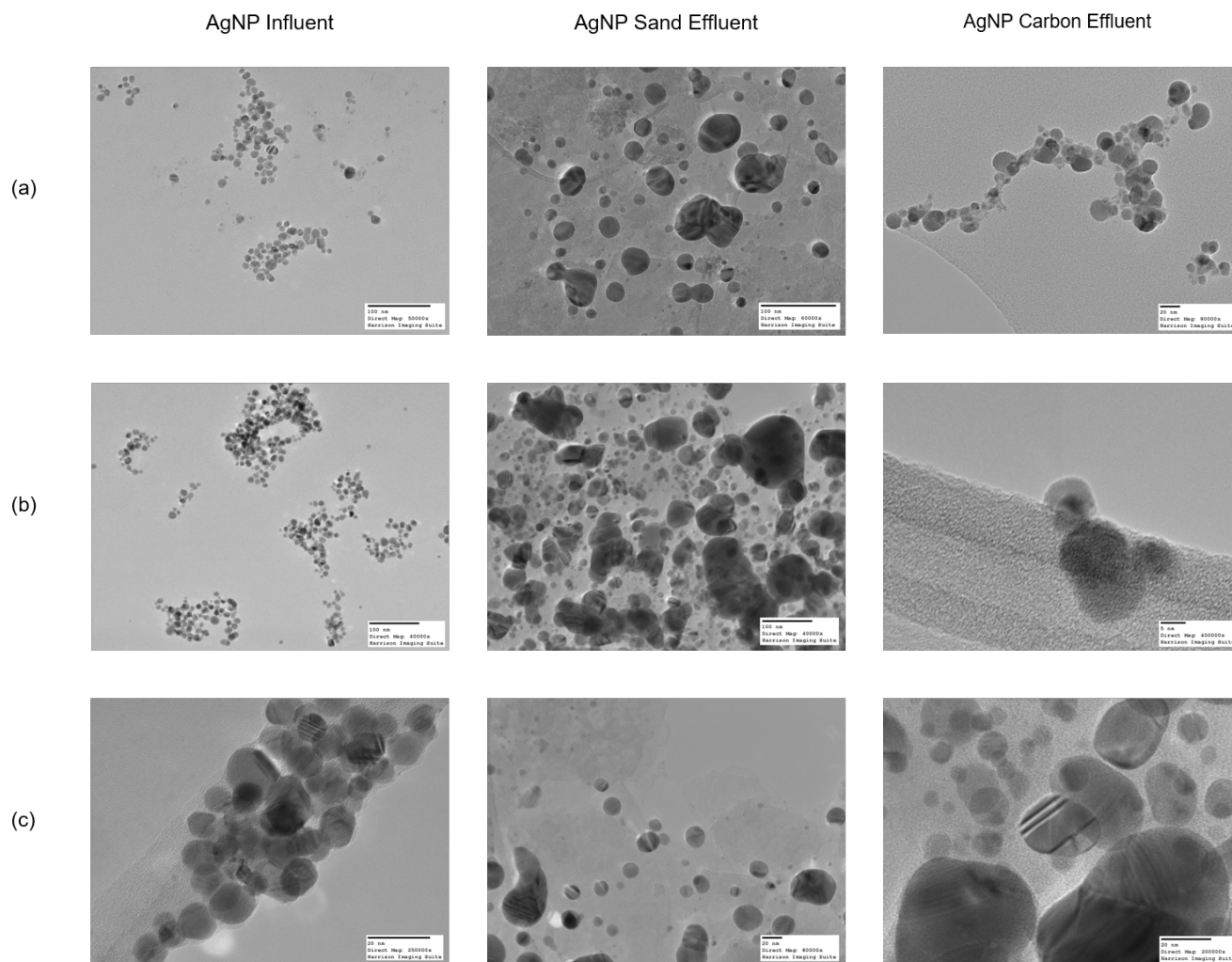


FIGURE 5.1: TEM Image of AgNPs influent and effluent samples. In ascending order from left to right: AgNPs Influent (a), scale bar 100nm, direct magnification 50000x; AgNPs Influent (b), scale bar 100nm, direct magnification 40000x; AgNPs Influent (c), scale bar 20nm; direct magnification 250000x; AgNPs sand effluent (a), scale bar 100nm, direct magnification 60000x; AgNPs sand effluent (b), scale bar 100nm, direct magnification 40000x; AgNPs sand effluent (c), scale bar 20nm, direct magnification 80000x; AgNPs carbon effluent (a), scale bar 20nm, direct magnification 80000x; AgNPs carbon effluent (b), scale bar 5nm, direct magnification 400000x; AgNPs carbon effluent (c), scale bar 20nm direct magnification 200000x.

5.3.2 Particle size distribution analysis

For the particle size distribution (PSD) analysis, the AgNPs particle diameter data was first checked for normal distribution by calculating and comparing the mean, median and mode. Which under normal distribution conditions, should all be comparable.

Mean:

$$Mean = \frac{1}{n} \sum_{i=1}^n x_i$$

Median:

$$\text{Odd number of samples: } Median = \left(\frac{n+1}{2}\right)^{th} \text{ term}$$

$$\text{Even number of samples: } Median = \frac{\left(\frac{n}{2}\right)^{th} \text{ term} + \left(\frac{n}{2}+1\right)^{th} \text{ term}}{2}$$

Mode: *The most frequently occurring value.*

The results of the above preliminary analysis are presented in table 5.1. Based on this initial analysis, all three influents were identified as normal distributions; while all the effluents could be described as skewed. Histogram plots (Figure 5.2) all confirmed this as a positive skew (to the right) indicating an increase in particle size to varying degrees.

This positive skew was further confirmed by calculating the skew of each data set.

Skewness

$$\frac{n}{(n-1)(n-2)} \sum \left(\frac{x_i - \bar{x}}{s} \right)^3$$

This confirmed that all three quartz sand effluent AgNPs were highly skewed to the right, each with an average particle size of 17.63nm (sample a), 22.38nm (sample b), and 14.77nm (sample c). Contrary to the preliminary analysis, AgNPs influent (c) was also mathematically considered highly skewed. Carbon effluents gave mixed distribution results where AgNPs carbon effluent (a) was moderately skewed, (b) normal and (c) highly skewed.

The symmetry of the distribution was further investigated by calculating the Kurtosis of each data set. Kurtosis measures the tail-heaviness of the distribution. Kurtosis decreases as the tails become lighter and increases as they become heavier.

Kurtosis

$$\left\{ \frac{n(n+1)}{(n-1)(n-2)(n-3)} \sum \left(\frac{x_i - \bar{x}}{s} \right)^4 \right\} - \frac{3(n-1)^4}{(n-2)(n-3)}$$

Where s is the sample standard deviation.

$$\sqrt{\frac{\sum(x - \bar{x})^2}{(n-1)}}$$

Where Kurtosis is near to zero (zero excess of kurtosis), the distribution plot can be confirmed as Mesokurtic, which is included within the normal distribution family,

regardless of its parameters. This is true for AgNPs Influent (a) and (b). Which confirms an even particle synthesis around the mean particle size of 9.98nm. An AgNPs particle size that can be considered environmentally high risk.

Where there is a positive excess of kurtosis the distribution is described as Leptokurtic, which can be viewed in the histogram plots as fatter tails (positive skew), true for all the quartz sand effluent samples. Thus confirming their propensity to increase in particulate size and mass following exposure to the quartz sand media bed. On average it was confirmed that 83% of sand effluent AgNPs had increased in size up to a maximum of 144nm in diameter.

Carbon effluent c) also exhibited Leptokurtic symmetry, while the remaining two carbon effluent samples, a) and b) can be described as Platykurtic, which describes the presence of thinner tails. As PSD for carbon effluent AgNPs are also positively skewed, this confirms some size increase, but not to the same extent as their sand counterparts. On average 33% of the carbon effluent AgNPs increased in size, up to a maximum of 51nm.

In both cases the size increase can be considered an environmentally positive result as the AgNPs are by processes of agglomeration and aggregation showing evidence of increasing in mass, which proportionally presents a reduction in cytotoxicity risk. However, in the case of this study, the increase in average particle sizes was not significant enough to as to render them toxicologically benign. Where both quartz sand and carbon effluents have increased in average size to 18.26nm and 13.31nm respectively, they now fall outside of the 1 – 10nm risk category of particles able to attach to cell membranes or penetrate cell walls. Yet, can still be characterised as particles that fall below 20nm which are considered high risk in

aquatic environments due to high surface reactivity and increased capacity for silver ion release.

	Influent (a)	Influent (b)	Influent (c)	Sand Effluent (a)	Sand Effluent (b)	Sand Effluent (c)	Carbon Effluent (a)	Carbon Effluent (b)	Carbon Effluent (c)
Sample size	131	210	46	104	205	59	59	5	51.24889
Max (nm)	20.02	19.80	18.05	66.55	144.24	63.26	29.75	15.09	51.25
Min (nm)	3.76	4.15	5.39	3.60	2.93	2.20	3.98	7.65	0.42
Mean (nm)	9.45	10.66	9.82	17.63	22.38	14.77	14.70	11.31	13.94
Median (nm)	9.43	10.19	9.83	13.84	14.48	12.28	12.88	10.45	9.11
Mode (nm)	9.69	8.78	-	9.00	7.16	4.66	9.35	-	-
Kurtosis	0.26	-0.49	1.54	4.10	7.67	4.18	-0.37	-2.92	2.57
Skewness	0.55	0.40	1.54	1.99	2.18	1.74	0.69	0.26	1.78
STDEV	3.37	3.43	2.54	13.90	19.54	11.70	6.33	3.48	11.61
Distribution	Normal	Normal	highly skewed, positive	highly skewed, positive	highly skewed, positive	highly skewed, positive	Moderately skewed	Normal	highly skewed, positive
Symmetry	mesokurtic distribution	mesokurtic distribution	mesokurtic distribution	leptokurtic distribution	leptokurtic distribution	leptokurtic distribution	platykurtic distribution	platykurtic distribution	leptokurtic distribution.

TABLE 5.1: Table of particle size distribution properties for AgNP influents colloidal solutions and AgNP effluents resulting from quartz sand columns and GAC isotherm batch reactors.

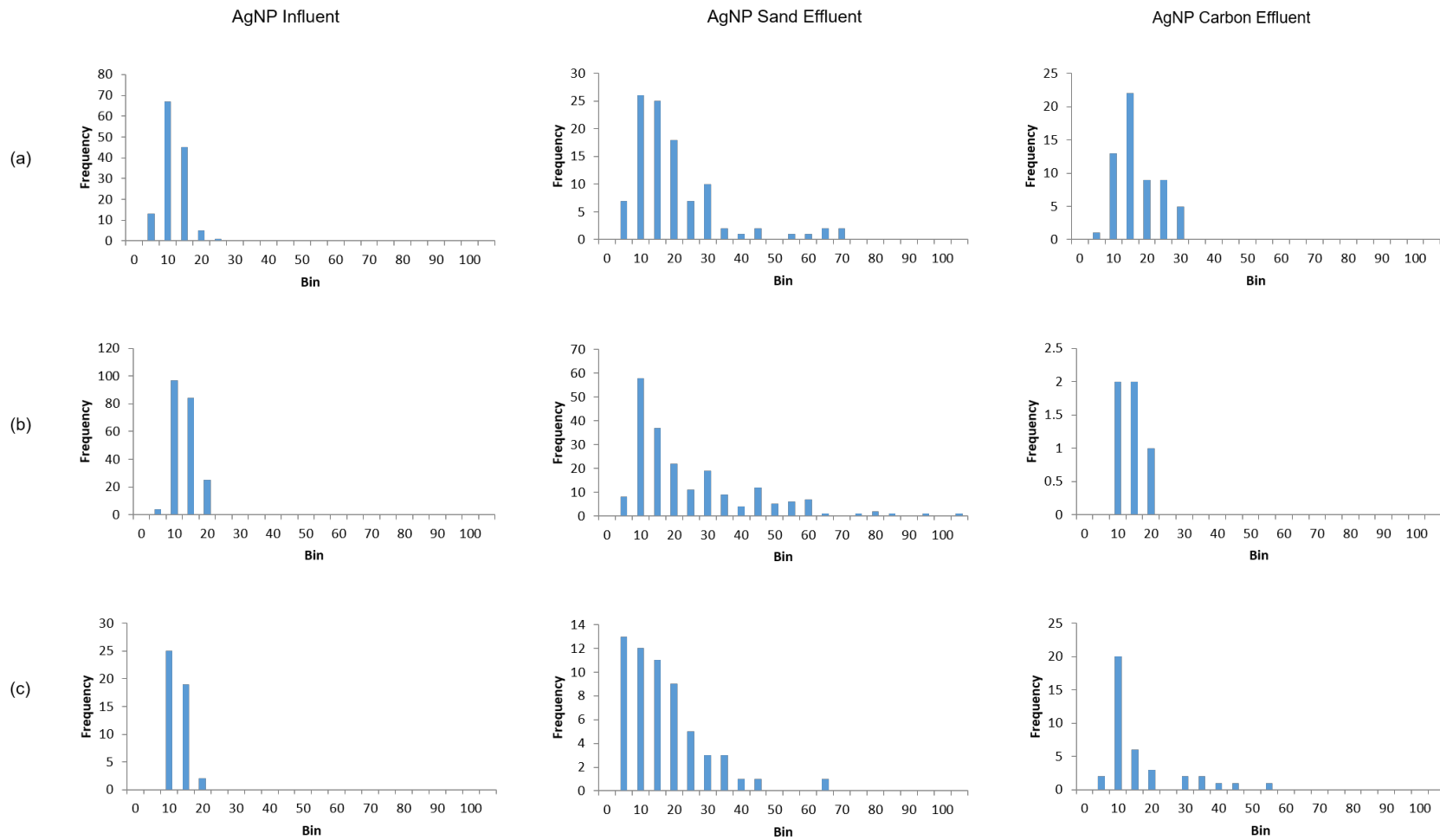


FIGURE 5.2: Particle size distribution histograms in descending order from left to right: AgNPs Influent (a), AgNPs Influent (b), AgNPs Influent (c), AgNPs sand effluent (a), AgNPs sand effluent (b), AgNPs sand effluent (c), AgNPs carbon effluent (a), AgNPs carbon effluent (b), AgNPs carbon effluent (c).

5.3.3 EDS analysis of AgNPs influents and effluents.

AgNPs Sand Effluent

As is evident in the TEM images (Figure 5.1) AgNPs Sand Effluent (a), (b) and (c), the AgNPs have not just increased in size, but also appear to have collected surface coatings and residues. It is likely that these surface coatings and residues have contributed to and / or promoted the physical and chemical processes of aggregation and agglomeration.

The EDS analysis of the AgNPs sand effluent sample showed a considerably more complex elemental characterisation of the sample than that of the influent or carbon effluent EDS (Figure 5.3). Two EDS elemental analyses were taken of the AgNPs sand sample from different positions on the TEM grid. Against the backdrop of C and Cu, the Quartz sand effluent EDS images b) and c) indicate the additional presence of Iron (Fe), and Chromium (Cr) to a lesser degree and a significant presence of Gold (Au). The composition of the quartz sand as purchased was described as 96.2% SiO₂; 1.4% Al₂O₃; 0.1% Fe₂O₃; 0.04% CaO; 0.04% Na₂O; 0.02% MgO. The presence of Fe (Figure 5.3 image (c)) can be explained by the (as sold) composition of the sand. The presence of Cr remains unexplained, but as it was relatively minimal this was left un-investigated. The Au contamination was however considerable and may be attributed to being naturally occurring with the sand media, where there is a known geological association of gold with quartz; where gold veins commonly, but not inclusively, form within quartz rock (Zhu et al. 2011).

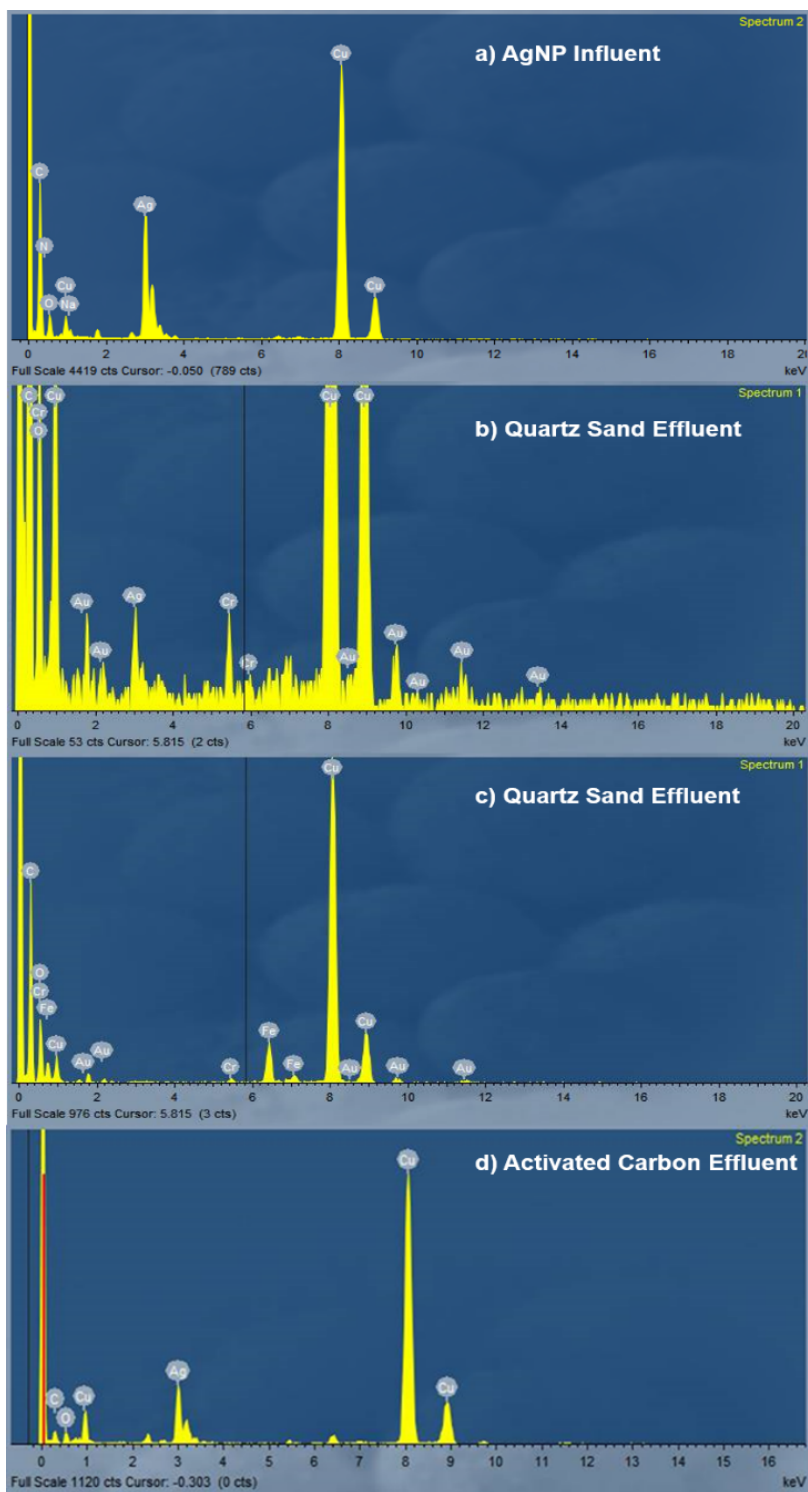


FIGURE 5.3: EDS elemental analysis of AgNPs effluents. a) AgNPs Influent, b) Quartz sand effluent, c) Quartz sand effluent, d) Activated carbon effluent.

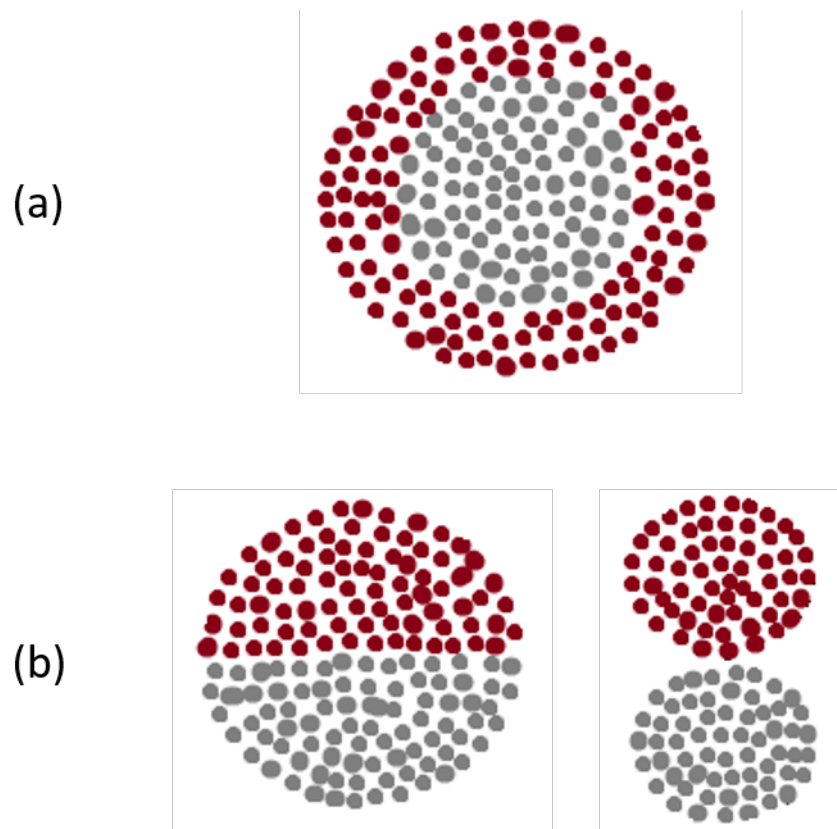


FIGURE 5.4: Schematic representation of possible alloy cluster mixing patterns, adapted from Ferrando et al, 2008. (a) core-shell, (b) sub cluster segregation.

Sand sourced from coastal regions may also have been subject to ocean water gold contamination, where it is estimated that oceans waters contain nearly 20 million tons of gold, either as dissolved complexes, colloids, nano-particles, aqueous clusters, absorbed onto detrital clays and as Au–organic complexes (Large et al. 2015). Nano alloy clusters, such as silver–iron and silver–gold can be formed and identified via several mixing patterns. Core-shell segregated nano alloys (Figure 5.4 (a)) consist of one type of atom (the core) surrounded by a (shell) coating of another., or sub cluster segregated nano alloys (Figure 5.4 (b)) which consist of a mixed interface of atom bonds (Ferrando et al. 2008). In the case of silver–gold alloy nanoparticles, it has been observed that the

presence of gold has a deactivating effect on silver ion release, significantly reducing the antibacterial and cytotoxic effect of the AgNPs (Grade et al. 2014).

The carbon effluent samples were less affected, appearing to only change in size / mass rather than be chemically affected by the GAC media environment. This can be attributed to the fact that GAC as a powerful adsorbent, is effective in adsorbing and containing any additional contaminants / ligands in the supernatant, thereby rendering them unavailable to the AgNPs. The EDS analysis confirms this by demonstrating a simple elemental characterisation of AgNPs effluent, against the expected backdrop of C and Cu (Figure 5.3 (d)). In the literature it is evident that some focus has been given to the manufacturing of AgNPs doped activated carbon for enhanced water purification technologies, observed to improve GAC adsorption capacity for certain dyes (Yellow 12 and Methylene Blue) (M. Ghaedi et al. 2012; Mehrorang Ghaedi et al. 2012) or to improve methods of water disinfection and purification (Lalley et al. 2014; Li et al. 2008; Park & Jang 2003; Pal et al. 2009).

5.4 Limitations

TEM and EDS analysis was not run for effluent samples that were known to not contain AgNPs. As a result the study as described in this chapter lacks a control sample for both quartz sand and GAC. As such it is not possible to definitively claim that the particles as depicted in the TEM images are AgNPs. Evidence supporting the presence of silver and AgNPs is derived from EDS elemental analysis and from the previous photometer, ICP-MS and ICP-OES that these effluent samples had been subjected through

5.5 Conclusion

The objective of this work was to study particle size distributions of AgNPs resulting from exposure to quartz sand and activated carbon media beds. Several PSD analysis techniques were used to investigate how the AgNPs were affected following transport through their respective media beds. Conclusions from the present study are as follows.

- i. AgNPs synthesis achieved a relatively homogeneous dispersion of AgNPs average particle size of 9.98nm and spherical in shape. This homogeneous dispersion is described by a normal Mesokurtic PSD and is in keeping with the characteristics of AgNPs commonly used in many domestic and commercial applications.
- ii. AgNPs carbon effluent sampled exhibited a propensity to Platykurtic PSD, whereby only 33% of the sample was observed to change / increase in particle size, with a total average size of 13.32nm. This growth in size is not significant enough to mediate concerns regarding AgNPs toxicity in either WwTP or natural environments.

- iii. AgNPs sand effluent samples were considerably more heterogeneous in nature, exhibiting leptokurtic PSD. 83% of the sample changed / increased in size from an average of 18.26nm up to a maximum of 144nm. The increase in average size for 83% of the sample is encouraging, in that it renders the particles larger than the size category (1 – 10nm) of direct cytotoxicity, via cellular membrane attachment or cell penetration. Yet with the majority of the size distribution falling at or under 20nm, there remains relevant concerns for their toxicity potential in the aquatic environment, due to properties such as high surface reactivity and ion release.
- iv. The conservative and somewhat homogeneous growth of the AgNPs carbon samples can be attributed to the powerful adsorbent action of the GAC, which by readily adsorbing any additional contaminants or ligands within the liquid system, renders them unavailable to the AgNPs. As the AgNPs are adsorbed in a relatively unchanged state, they may however donate their unique nano properties to the carbon matrix and aid improved treatment performance for both the adsorption of other materials and by way of added disinfection abilities.
- v. The heterogeneous nature of the AgNPs sand effluent can be attributed to both physical and chemical processes of agglomeration and aggregation in response to exposure to a range of residual compounds and ligands present within the quartz sand media bed; particularly for the potential to form nano alloy clusters with iron and gold. With respect to the formation of gold silver nano alloy clusters, it has been observed in the literature that the presence of gold in the structure deactivates the AgNPs capacity to release silver ions. Thus as a result of their transport

through the quartz sand and their association with residual gold to form nano clusters may lead to more positives outlooks and fates for AgNPs in the aquatic environment.

CHAPTER 6

CONCLUDING DISCUSSION AND FUTURE WORK

6.1 Concluding discussion

Silver nanoparticles (AgNPs) due to their unique and technologically advantageous characteristics such as unique optical, conductivity, thermal and mechanical properties have become widely employed across several sectors of industry, medicine, electronics and technology (Klaine et al. 2008). In addition, the popularity and demand for their use across a wide range of high street consumer products is continuously growing, with new products arriving on the market almost daily (Murphy et al. 2015; Woodrow Wilson International Center for Scholars 2013; Walters et al. 2014; Vance et al. 2015). The bactericide properties of silver ensure the continued appeal for AgNPs inclusion in cosmetics, soaps, socks, clothes, detergents, keyboards, medical bandages and wound dressings (Benn & Westerhoff 2008; Colman et al. 2014). The fate of these silver nanoparticles is, however, less clear as their disposal and release depend largely on consumer's habits (Gupta et al. 2015; Murphy et al. 2015; Maynard et al. 2006).

Since a large proportion of products using silver nanoparticles are textile, cosmetic and personal hygiene, the expected release of AgNPs will occur during normal washing, rinsing and hygienic labours. These AgNPs travel to our wastewater treatment plants, estimated at an influent loading of 8 t/year of AgNPs in UK in 2013 (Whiteley et al. 2013), with wider projections for 190-410 t/year for Asia, North America and Europe made in 2014 (Siripattanakul-Ratpukdi & Fürhacker 2014). Following entry to WwTP, effluent emissions of AgNPs are expected to reach the environment. At point of discharge the levels of AgNPs have been suggested at 0.003-0.26 µg/L for fresh waters, with higher concentrations expected to be contained with wastewater treatment sludge

solids (Lazareva & Keller 2014). Although, the magnitude of the effects of these AgNPs in the environment is not clear, existing literature suggests negative effects in all organisms evaluated to date (from bacteria to mammalian cell lines). The proper and accurate evaluation of these AgNPs releases on the environment is, however, not a straight forward task. Interacting processes such as agglomeration, dissolution, chelation, sedimentation, oxidation and animal and plant uptake are some of many the processes that complicate the evaluation of the AgNPs effects and consequences in natural matrices.

The concern for the mobility of AgNPs from point of use to environmental sink is twofold.

The principal route for AgNPs entry into the aquatic environment is via WwTP (Siripattanakul-Ratpukdi & Fürhacker 2014; Levard et al. 2012; Colman et al. 2014). Thus the first concern is regarding the potential for AgNPs to negatively affect the functional processes and efficacies of microbes in wastewater treatment facilities (Brar et al. 2010). Furthermore, the capability of treatment processes to effectively remove these nanoparticles from wastewater streams is not well understood or quantified (Brar et al. 2010).

The second key concern is for the fate of AgNPs released into the environment from WwTPs and the potential for toxicity to a wide range of organisms (Webb et al. 2001; Sathya et al. 2012; Howe et al. 2002). However, an accurate evaluation of AgNPs environmental risks in natural compartments is not an easy task due to:

- a) the complex array of physical, biological and chemical processes present in the natural environmental setting,
- b) a limited understanding of the true concentrations and physical and chemical characteristics of the WwTP AgNPs emissions, and
- c) current limitations in our ability to capture, identify and quantify AgNPs in natural matrices due to a range of sampling and measuring constraints such as high level of sample manipulation prior to testing being required, or quantification techniques that are unable to differentiate between silver nanoparticles, ions or other silver complexes.

Some of the key knowledge gaps that require attention include,

- a) The requirement for more comprehensive data on releases of AgNPs into wastewaters and receiving natural waters, including validated empirical data for real world AgNPs concentrations.
- b) Comprehensive studies that seek to investigate the fate and transport of AgNPs through all WwTP compartments, including methods of filtration and clarification. Data from such studies, if approached in a logical manner that seeks to start simply, but to then sequentially layer up the complexity of influencing factors, would be highly informative to the the development of comprehensive and sophisticated models for AgNP toxicity.
- c) An more informed understanding of AgNPs transformation and subsequent particle characterisation and / or chemical speciation under relevant wastewater treatment conditions, including an improved understanding of the resulting mobility and bioavailability of the transformed complexes.

- d) In support of the above a need for the improvement of analytical techniques to allow for the accurate detection, visualization, and quantification of AgNPs fate, behaviour and interactions in complex environmental samples, both in controlled laboratory settings, but also in field environments, investigating the occurrence and fate of AgNPs in a real world setting.

6.2 Conclusions

This study focused upon the fate and transport of AgNPs across two media types commonly used as filtration methods in wastewater treatment processes.

- It was found that AgNPs were highly dynamic and mobile through quartz sand media beds, with c. 100% recovery at point of discharge. Where sand filtration is employed in water treatment plants either as a slow sand filtration method or, more notably, as a tertiary process of clarification, there is a reasonable risk that the mass of AgNPs entering the system would also exit the system.
- The high mobility observed in this study, was attributed to repulsion forces between the negatively charged silica surfaces of the quartz sand and AgNPs that had acquired a negative surface charge, as a result of negative boride ion contamination from the synthesis process. This serves to emphasise the importance of fully understanding the effects that various AgNPs synthesis methods and the application of various capping agents have on the transport fates of AgNPs.
- It was also found that AgNPs are significantly affected by their transport through the quartz sand environment, resulting in a high degree of agglomeration and aggregation of up to 83% of the influent mass.

- This high degree of agglomeration and aggregation could be expected to have a positive influence on the environmental fate of the AgNPs, in that the quartz sand environment clearly promotes particle size growth, thus exacting a positive influence towards reducing the supposed toxicity risk as linked to size.
- However, in this study the average growth measured for AgNPs, while theoretically sufficient to reduce the potential for direct cytotoxicity, was not significant enough to reduce more general means of toxicity for the majority of aquatic organisms.
- The growth in size of AgNPs following transport through the quartz sand media can also be attributed to the formation of silver alloy nano clusters as a result of both physical and chemical processes, to which the range of residual compounds and ligands, such as gold, naturally present within the quartz sand contributed to. In the case of silver-gold nano alloy clusters, it has been observed in the literature that the presence of the gold in the cluster has a deactivating effect on the AgNPs potential to release silver ions, thereby reducing much of the inherent toxicity potential.
- What this study did not take into account was the inclusion of any naturally occurring biofilms within the quartz sand media bed, which are commonly encouraged to form in WwTP sand filtration systems; or the use of pre-treatment polymers, which are often employed to promote the coagulation and flocculation of suspended particles within the wastewater liquor. It would be expected that the inclusion of these factors, would promote AgNPs retention within the sand filter environment, though results would be expected to vary for colloidal

stability, mobility and chemical reactivity depending on the method of AgNPs synthesis and capping agents applied. However, it should be noted that the removal of AgNPs from the liquid emissions of WwTP does not necessarily negate the ultimate environmental fate of AgNPs, but instead would transfer the concern to the WwTP sludge solids emissions, which are commonly employed in agriculture as a fertiliser or sent to landfill. From both these end-points AgNPs may still navigate into natural water (including groundwater) sources by way of surface run off and soil leaching.

- With regards to the adsorption of AgNPs to activated carbon media, it was confirmed that activated carbon was an effective adsorbent for AgNPs.
- Although, with regards to silver in general, it appears that particle size is an important factor in this process; where it was found that AgNPs of average particle size 9.98nm were more effectively adsorbed onto the carbon, while the bulk silver comparison of particle sizes 300 – 800nm were not adsorbed.
- It is noted though that the literature suggests that the adsorption of bulk silver onto activated carbon is low pH, high salt dependant process, additional factors which this studies did not take into account. It was also noted, that AgNPs exposure to carbon did not have a significant impact on the particles physical or chemical characteristics. Where only 33% of the sample increased in average particle sizes from 9.98nm up to 13.32nm.
- This indicates activated carbon as an effective treatment process for the removal of AgNPs, particularly so as a final clarifying process.

- In addition, it is possible that the adsorption of AgNPs onto the surface of activated carbon may indeed promote improved activated carbon wastewater treatment performance, by either enhancing its' ability to adsorb other material contaminants or by contributing an additional antimicrobial action to the filtration process (Rananga & Magadzu 2015).

As part of the development of this study, a simple, novel laboratory technique was established and employed for the measurement of AgNPs concentration in laboratory samples.

- This technique used a bench top photometer, for which the underlying principle is based upon Beer-Lamberts Law, in which the light absorbance of a solution is considered to be directly proportional to the concentration of the absorbing species in solution and the path length (Miller et al. 2009).
- The accuracy of this method was validated by ICP-MS and ICP-OES and it was found to be effective for laboratory samples between a detection range of 0.01 and 20mg/L (~20 mg/L being the upper limit of the experimental procedure).
- It is proposed that following further development and refinement of this technique, that this may provide a useful and quick support tool for laboratory researchers in the quantification of AgNPs in aqueous samples.
- This method might also be interest to end-users in industry and environmental law enforcement and monitoring agencies as an indicative, AgNPs specific, quantification tool that yields fast, low cost results, pending further laboratory ICP-MS / OES confirmation later.

6.3 Recommendations for Future work

In support of the development of multiscale algorithms and models that provide accurate information regarding the real world structure and dynamics of nanoparticles in complex environments, a considerable breadth and depth of data is still required. At a molecular scale there is a need to further explore how AgNPs are chemically and characteristically influenced by the various urban and environmental compartments that they are exposed to, from point of use to environmental sink. At larger scales, models must be capable of coping with the changing behaviour and transport of AgNPs within various environmental compartments and inside organisms. Continued column studies for fate and transport studies can help contribute to this effort by further investigating:

- a) The mobility, particle fate and behaviour of transformed, aged and recycled AgNPs and AgNPs clusters and complexes through all aspects of wastewater treatment processes, both as isolated units and in sequence reflecting the true WwTP life cycle.
- b) Where appropriate to do so, include the use of pre-treatment polymers and chemicals to assess the impact that these additives may have on rates of agglomeration and aggregation, and how this influences mobility and particle characterisation.
- c) Synthesize backwash and desludging simulations on the laboratory columns and batch reactors to investigate and quantify the potential for AgNPs to be transferred from the wastewater liquor into wastewater treatment sludge's from all steps in the wastewater treatment process.

With regards to environmental toxicology investigations, clear information and data is required regarding the range of expected concentrations, particle characterisation and speciations of AgNPs resulting from urban and WwTP environments. Where parameters such as solubility, zeta-potential (colloidal stability), aggregation, agglomeration, and / or change in Ag speciation will have a significant impact on its reactivity with different species present in aquatic systems. Such data should:

- a) Widen the scope for characterisation of AgNPs, to ensure that environmentally relevant parameters such as surface charge, dissolution rate, surface area, Z-Average, zeta potential, aggregation and chemical speciation. In particular, the dissolution rate of the resulting Ag / AgNPs complexes is considered a highly valuable parameter for further environmental fate and eco-toxicological studies.
- b) Employ AgNPs influent and effluent concentrations that better reflect the current estimate of 0.003-0.26 µg/L effluent discharge concentrations, to ensure that real world relevant WwTP exposure and emission data is achieved.

To further understand the potential for risk of AgNPs in WwTP and environmental compartments improved sampling, quantification techniques must be developed in order to detect, quantify, and characterise AgNPs in natural matrices. Key factors to consider in the development of such techniques include reducing the requirement for sample manipulation prior to testing and to ensure that quantification techniques can distinguish between different Ag

speciations, including nanoparticle variations. The photometer method as outlined in Chapter 3, showed some limited promise in this area. To further test the robustness and future potential of this method as a low tech option in support of the above the following would need to be explored:

- a) Establishing light absorbance and concentration curves for a range of AgNPs sizes and crystalline structures.
- b) Investigate the influence of commonly employed capping agents on the optical properties of AgNPs and the photometer capability to be sensitive to these changes in optical properties.
- c) Calibrating and validating the established concentration curves for a range of environmentally relevant mediums in which AgNPs are suspended / contained.
- d) Investigate the influence of interactions with environmentally relevant ligands upon AgNPs optical properties.
- e) Working within improved and increased ranges for absorbance and transmittance thereby increasing measure accuracy but also the capability to detect and quantify AgNPs at more environmentally relevant concentrations

CHAPTER 7

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