TAMPERE UNIVERSITY OF TECHNOLOGY

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SILVER DISSOLUTION AND FUNCTIONALITY OF SILVER
DOPED SUPERHYDROPHOBIC SURFACES

Master of Science thesis

ABSTRACT

Silver is highly toxic for microorganisms but harmless for humans. Silver is used in many different applications to reduce and prevent microbial growth. In this thesis dissolution of silver in aqueous environments and the effect of dissolved silver on bacteria have been studied. This work describes also other properties such as superhydrophobicity and photocatalytic property that could prevent bacterial growth. The possibility of combining antibacterial silver, photocatalysis and superhydrophobicity to enhance the antibacterial effect has been reviewed and tested.

Superhydrophobic coatings doped with silver particles were prepared and the solubility of silver was determined by atomic absorption spectrometry (AAS). Silver dissolution from commercial silver nanoparticles in alkaline, acidic and neutral solutions was also studied. The functionality of the prepared superhydrophobic coatings was examined by measuring water contact angle after exposure to alkaline, acidic and aqueous environment. The antibacteriality of silver containing superhydrophobic coatings was tested by VTT (VTT Technical Research Centre of Finland).

The results showed that silver dissolves in all environments tested. The solubility rate in alkaline and acidic environment is higher than in pure water but the amount of dissolved silver was high enough to cause antibacterial effect in all environments. However, the durability of the coatings in constant exposure to the solutions was relatively poor.

The results of this work show that the prepared silver containing superhydrophobic coatings have potential to be used in antibacterial applications when they are not exposed constantly to aqueous environment. The durability of the coatings could possibly be improved by choosing different materials for the coating and modifying the structure. Surfaces that combine superhydrophobicity, photoactivity and silver for antibacterial applications are currently under development.

TIIVISTELMÄ

Hopea on tehokas ja yleisesti tunnettu antimikrobinen metalli. Hopeaionit tuhoavat ja estävät lukuisten eri mikro-organismien kasvua, mutta ovat harmittomia ihmiselle. Tässä työssä on tutkittu hopean liukenemista happamassa, emäksisessä ja neutraalissa ympäristössä. Työssä kuvataan myös superhydrofobisuuden ja fotoaktiivisuuden vaikutusta antibakteerisuuteen.

Tutkimusta varten valmistettiin superhydrofobisia pintoja, joiden päälle lisättiin hopeapartikkeleita. Hopean liukenemista valmistetuista pinnoista ja kaupallisista hopeananopartikkeleista mitattiin atomiabsorptiospektrometrian (AAS) avulla. Pintojen kestävyyttä tutkittiin mittaamalla veden kostutuskulma pinnoista, jotka olivat olleet jatkuvassa altistuksessa tutkittaville liuoksille. VTT (Teknologian tutkimuskeskus VTT) testasi pinnoitteiden antibakteerisuutta.

Tulokset osoittavat, että hopeaa liukenee kaikissa testatuissa olosuhteissa riittävästi antibakteerisen vaikutuksen aikaansaamiseksi. Kostutuskulman pieneneminen kuitenkin osoitti, että pinnoitteet eivät kestä jatkuvaa altistusta happamalle, neutraalille tai emäksiselle vesiliuokselle.

Tulosten perusteella voidaan todeta, että valmistetut superhydrofobiset, hopeaa sisältävät pinnoitteet, estävät tehokkaasti bakteerien ja mikro-organismien kasvun. Pintojen kestävyysongelmat aiheuttavat kuitenkin rajoituksia käyttömahdollisuuksille. Jatkotutkimuksen kohteena on paitsi antibakteerisuuden kehittäminen, myös pintojen paremman kestävyyden aikaansaaminen. Myös mahdollisuus yhdistää hopean antibakteerisuus, superhydrofobisuus sekä fotoaktiivisuus on tutkimuksen kohteena.

PREFACE

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Contents

Abbreviations

AAS	Atomic absorption spectroscopy
AFM	Atomic force microscopy
Ag	Silver
Ag^+	Silver(I) ion
AgBr	Silver bromide
AgCl	Silver chloride
AgI	Silver iodide
$AgNO_3$	Silver nitrate
Ag_2S	Silver sulphide
$(Al(NO_3)_3)$	Aluminum nitrate
Al_2O_3	Aluminum oxide
ATP(ases)	Adenosine triphases
BSE	Back-scattered electron detector
CF_3	Carbon trifluoride
CellC	Automated image analysis software
C_2H_5OH	Ethanol
$C_{12}H_7NO_4$	Resazurin
CVD	Chemical vapour deposition
DNA	Deoxyribonucleic acid
E	Energy
EDS	Energy dispersive spectroscopy, Energy dispersive x-ray analysis
FAS	(heptadecafluoro-1,1,2,2-tetrahydrodecyl)trimethoxysilane
FESEM	Field emission scanning electron microscopy
HCl	Hydrochlorid acid

HNO_3	Nitric acid
H_2SO_4	Sulphuric acid
ICP-MS	Inductively coupled plasma mass spectrometry
ISA	Ionic strength adjustor
ISE	Ion selective electrode
SEM	Scanning electron microscopy
SYTO9	Green fluorescent nucleic acid stain
TiO_2	Titanium dioxide
TDS	Total dissolved solid meter
UV	Ultra violet
UVA	Ultraviolet A, radiation wavelength range 315- 400 nm
XRD	X-Ray diffraction
α- alumina	Thermodynamically stable phase of Al_2O_3
γ- alumina	Metastable Al_2O_3 structure
δ- alumina	Metastable Al_2O_3 structure

1 Introduction

Silver (Ag) has been known for its antimicrobial properties since ancient times. Two thousand years ago it was used in Egypt in long-term water storage and in the 900s the Chinese emperors used silver to prevent poisoning. Today antimicrobial properties of silver are exploited and researched more than ever. [1]

Antibacteriality of silver is most efficient when it is in ionic form (Ag^+) and therefore it is important to understand the dissolution mechanism to control the antibacteriality. There are several factors affecting the dissolution of silver including temperature, pH of the environment and composition of the solution. Some ions form permanent or poorly soluble compounds with silver decreasing the antibacterial properties. [2,3]

Besides silver there are also other materials known for their antibacterial properties. One of these materials is titanium dioxide (TiO_2). TiO_2 is semiconductor and strong photocatalyst. When titania is exposed with ultraviolet (UV) light in proper environment it forms radicals which have the ability to degrade organic molecules. It has been proposed that addition of silver particles on titania surface could increase the antibacteriality by decreasing the rate of electron-hole recombination reactions in the photoactive titania. In addition, the antimicrobial properties of silver itself increase the antibacterial effect of the system. [4,5,6]

Superhydrophobicity is a surface property that may possibly increase the antibacteriality. Superhydrophobic surface has a high contact angle with water and a low roll-off angle so that water will form pearl-like drop and roll-off easily when the surface is tilted a little. At the same time superhydrophobic surface requires surface roughness and low surface energy. When a water droplet rolls on a superhydrophobic surface the dust adheres to the water droplet and the surface is cleaned. This property is known as self-cleaning effect and it is exploited in many products. [7,8] Superhydrophobic surface is believed to lead to a low bacteria attachement possibility and also weak adhesion to the surface.

There are some products in which the superhydrophobicity and the antibacteriality of silver have been combined by adding silver particles on superhydrophobic surface. For example in superhydrophobic antibacterial cotton textiles these properties have successfully been combined. However the possibility of combining the three elements of photocatalysis, silver and the superhydrophobicity has rarely been realized. [9]

This work is related to TEKES funded Disconnecting project.

2 Dissolution of silver

This chapter describes properties of silver and the affecting mechanism by which silver inhibits bacterial growth. Dissolution is essential for antibacterial action of silver and therefore different mechanisms to determine the amount of dissolved silver are presented.

2.1 Antibacteriality of silver

The most common form of silver in nature is silver sulphide Ag_2S. However, there is also silver in metallic form (Ag). Silver does not oxidize in air and it is insoluble in some dilute acids such as HCl and H_2SO_4 but it dissolves in nitric acid (HNO_3) and cyanide solutions. The most common oxidation state of silver besides metallic silver is monovalent silver Ag^+. Silver appears also in bivalent and trivalent form. Silver forms compounds with halogens which are called silver halides (AgCl, AgBr, AgI). These compounds are poorly soluble. Silver forms permanent complex also with ammonia and cyanide ion. Some silver compounds such as AgF and $AgNO_3$ are highly soluble in water. [10]

Silver is highly toxic to a wide variety of micro-organisms. This antibacterial activity of silver has been known for thousands of years. Due to the introduction of antibiotics in the last century the use of silver in medicine decreased since the antibiotics are highly effective to kill bacteria. However, due to the negative impact of antibiotics to the environment and the restriction of law the applications of silver have attracted more attention in research and development [11]. From all forms of silver, ions have the strongest capability of destroying microbes. However, some other forms of silver function also as bactericide. Silver chloride for example is used in certain antimicrobial applications. [12]

Colloidal silver is widely used as a bactericide. Colloidal silver consists of silver particles dispersed in water. These silver particles are nanometric scale and have a high specific surface area [11,13]. These properties as well as the shape of the particles are believed to influence toxicity of colloidal silver [14]. To preserve the stability of the protective colloids, water soluble macromolecules must be used. Dispersed nanoparticles tend to display electronic and optical properties. Silver colloids absorb electromagnetic radiation (visible range) with distinct peaks. The wavelength of absorbed radiation depends on the particle size. [12]

It has been shown that the toxicity of Ag nanoparticles depends on the size of the particles at low concentrations. It also seems that the toxicity is dependent on the

shape of the particles. [14] Electron microscopy images of silver nanoparticles in bacteria containing environment are shown in Figure 1.
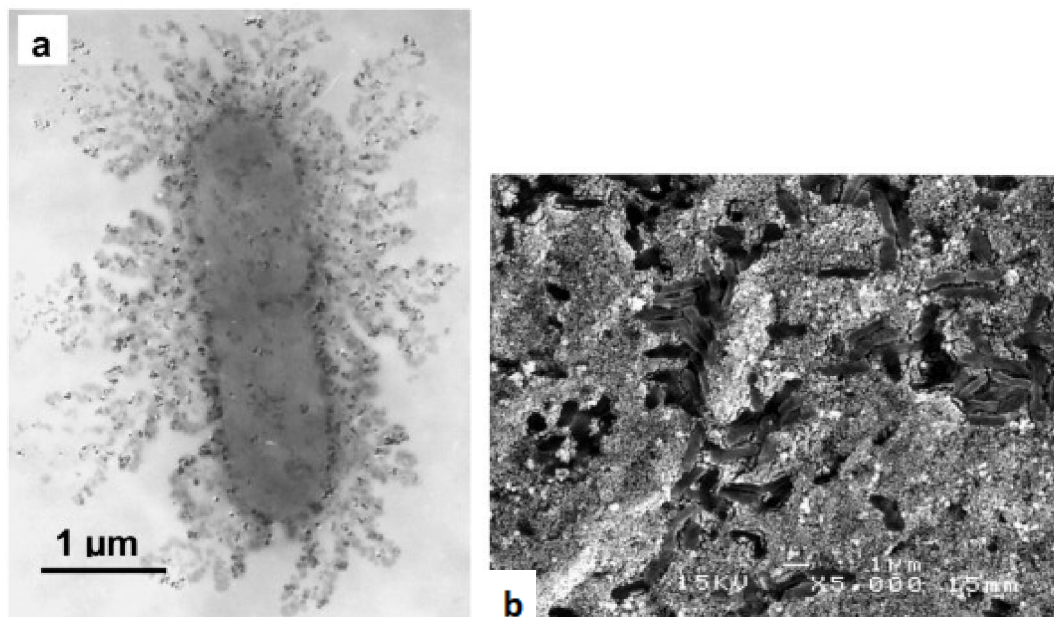
Figure 1. a: Escherichia Coli cell in liquid media containing silver nanoparticles (transmission electron paragraph). b: Bacteria (dark areas) and nanoparticle aggregates (SEM paragraph). [15]

The advantage of silver is that despite it is extremely toxic to a wide variety of bacteria it does not cause any side effects or damage to the human body excluding the nerve cells in the brain when silver has a direct contact to brain tissue [16]. It has been reported that silver also causes growth stimulation of injured tissue [17,18].

Silver resistant bacteria as well as silver bioaccumulation by microbes have been reported but the relationship between accumulation and resistance is not clear. The silver resistant bacteria have certain types of functions of silver resistance genes. Silver resistant bacteria have been found in environments such as burn wards of hospitals. The uncontrolled use of silver products should be avoided to prevent more bacteria from developing resistance. [16]

Antimicrobial activity is exploited to reduce infections and prevent bacteria colonization in various applications, for example dental materials, prostheses, stainless steel materials and textile fabrics. Silver can be used for water treatment and in medicine in burn treatment and for several non-medical applications such as antimicrobial protection of toilet seats, children's toys, telephone receivers and sports fabrics. [2,19]

2.2 Affecting mechanism on bacteria

There are three mechanisms by which silver may react with micro-organisms. Micro-organism may be destructed by oxidation catalysed by silver, bivalent or trivalent silver may cause the destruction of bacteria or monovalent silver may disrupt the electron transfer in bacteria. The monovalent silver may also substitute hydrogen ions and thus prevent the unwinding of DNA in viruses. [16]

However, the exact mechanism of the destruction of the bacteria is not well-known. It is believed that silver (I) is absorbed and accumulated in bacterial cells leading to shrinkage or detachment of the cytoplasm membrane. As a result DNA molecules lose their ability to replicate. Silver (I) may also block and inactivate proteins by interacting with their S-H bounds. [13]

Atomic silver adsorbs oxygen on to the surface. Oxygen diffuses easily within silver compared to other metals due to low repulsion of silver atoms. Molecular oxygen is converted to nascent oxygen by silver. It seems apparent that this behaviour with oxygen plays an important role in mechanism that causes antibacterial properties of silver. [16]

The metabolism of bacteria is destructively affected by silver ions at concentrations of 0.01–0.1 mg/l [12]. Therefore, some less soluble silver compounds, such as silver chloride (AgCl), also act as bactericides [12].

Silver resistant bacteria have repeatedly been reported. Ag(I) resistance results from energy-dependent ion efflux pumping from the cell by membrane proteins [2]. Ag(I) resistance system has two types of efflux pumps; those in which proteins function as adenosine triphases (ATPases) and those in which proteins functions as chemiosmotic cation/proton antiporters. [2]

2.3 Dissolution mechanism

Controlling the concentration of silver ions is one of the main requirements for silver used in bioactive coatings and biomedical applications [17]. Silver is relatively insoluble in pure water but it dissolves enough to exhibit a bactericidal effect [20,21]. One way to promote the dissolution is to maximize the specific surface area of the silver.

In the absence of halides Ag^+ ions are found to be the main product of the silver oxidation. In a solution containing Cl^- ions the porosity of formed AgCl layer affected the degree of Ag^+ formation. [17]

2.4 Environmental effects of silver

Organisms require small amounts of certain trace metals such as Cu, Fe and Zn for their functioning and growth. At higher concentrations these metals become toxic. Increased levels of background metals in coastal waters have caused negative consequences for ecosystem functioning. [22]

According to some studies silver is one of the most toxic metals for ecosystem functioning surpassed by only mercury [22-25]. Added to that silver is bioaccumulated by phytoplankton, seaweeds and marine invertebrates [23]. The toxicity of silver depends on its total concentration and its speciation. [24, 26-29]

2.5 The determination of silver

There are several methods to determine the amount of silver in a solution. When choosing a method to determine silver, factors like composition of the solution, form of silver and estimated amount of silver in the solution should be considered. Some commonly used methods to determine the amount of silver are presented in this chapter.

2.5.1 Atomic absorption spectroscopy

Quantities of chemical elements in a sample can be measured with Atomic absorption spectroscopy (AAS). The technique measures the radiation that the chemical element of interest absorbs by reading the spectra that is produced when radiation excites the atoms.

Each chemical element has a specific amount of energy that is required for an electron to leave the atom and it is called ionization energy. Atom emits a photon with energy E when an electron moves between energy levels. Every atom has a specific configuration of electrons in the outer shell and thus every atom has a unique pattern of wavelengths that it absorbs. Energy is inversely proportional to the wavelength as the equation 1 presents. [30,31]

$$E_2 - E_1 = \frac{hc}{\lambda} \tag{1}$$

where E_2 and E_1 are the energy levels of the atom, c is speed of light, h is the Planck constant and λ is wavelength.

When atoms absorb visible or ultraviolet light they make transitions to the higher energy levels. The wavelength of the light that transmits trough the sample is measured and it is compared with the initial wavelength of the radiation. The changes in absorbed wavelengths are integrated by a signal processor. [30]

According to the Beer Lambert law in equation 2 the concentration of the analyte is directly proportional to the absorbance.

$$A = lg \frac{I_0}{I}$$
(2)

where A is absorption, I_0 is intensity of the light before transmitting trough the sample and I is intensity of the light after transmitted trough the sample. [31]

Usually a calibration curve is used to determine the concentration. The calibration curve is determined by measuring standard solutions of known concentrations. [30]

Atomic absorption spectroscopy can be used to determine the concentration of atomic elements that absorb wavelengths between 190 and 900 nm. If the sample to be measured is not in atomic form it has to be broken in to free atoms. The decomposition can be done using a flame or graphite furnace.

Atomic absorption spectroscopy (Figure 2) can be used to measure total silver or ionic silver in the sample. Metallic silver particles must be removed from the solution when ionic silver is measured. One possibility to remove particles is by centrifugation as the density of metallic silver is ten times higher than that of silver ions dissolved in water. To separate the particles from ions a 365 000 G-force centrifuge should be used with a dwell time of 890 seconds. [32]

Ionic silver measurement with AAS is very accurate compared for example to the ion selective electrode. The result achieved by this method is directly the concentration of the ionic silver. [32]
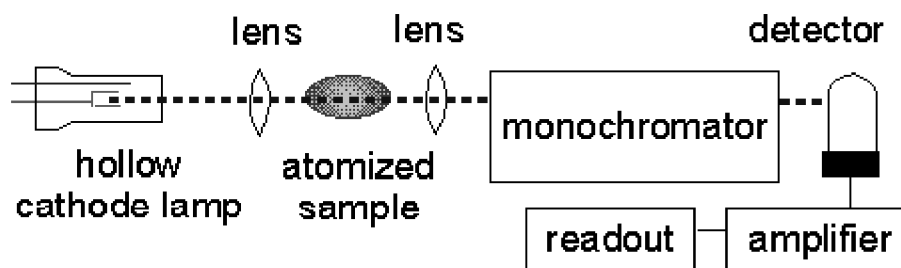
Figure 2. The structure of atomic adsorption spectrometer. [33]

2.5.2 Measurement with the ion selective electrode

An ion selective electrode (ISE) (Figure 3) responds to a specific ion, for example to a silver ion. The ISE must be calibrated before the measurement. Prior to the measurement the Ionic strength adjustor (ISA), usually sodium nitrate for silver ion electrodes, is added. After the addition of the ISA probe is immersed into the solution and it reads the concentration of silver ions in ppm. Only ions can be determined with this method. [32]

The accuracy of an ISE is usually within 2%. It shouldn't be used at concentrations below 0.01 ppm since the calibration is non-linear at that area [32].
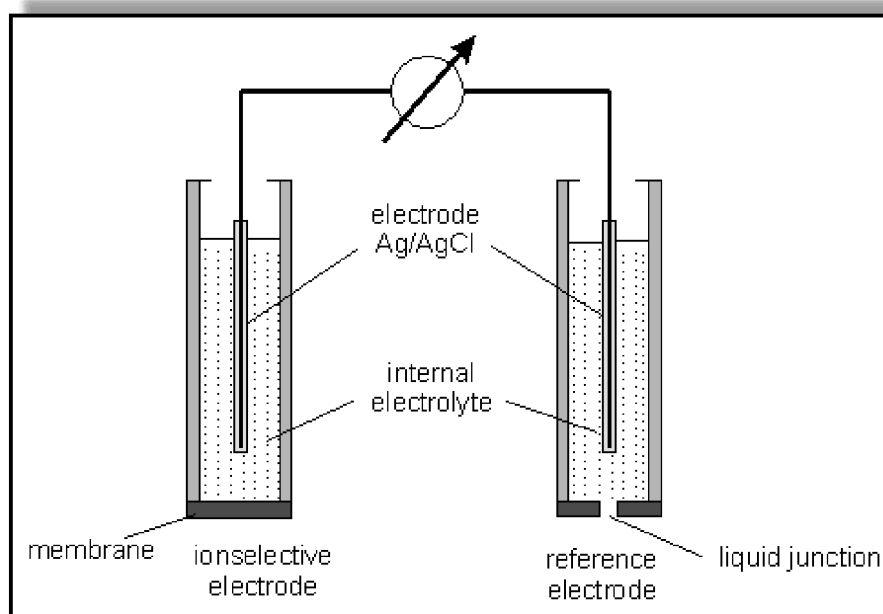
Figure 3. Ion selective electrode. [34]

2.5.3 Total dissolved solid metres

A total dissolved solid (TDS) meter measures the electrical conductivity of the sample and then multiplies it by a calibration factor. The result is expressed in ppm and all the ions in the solution affect it. The conductivity increases as the amount of ions in the solution increases. The accuracy of the result is the highest if there is only one type of ions without any impurity ions. [32]

In addition to the ion concentration there are also some other factors such as pH and temperature of the solution that have effect on the result. This must be taken into account when using conductivity measurement. [32]

2.5.4 Inductively coupled plasma mass spectrometry

Inductively coupled plasma-mass spectrometry (ICP-MS) (Figure 4) is a technique for chemical characterization of any material. It is suitable in many chemical analysis problems and it can be used in indentifying and measuring quantitatively any element in the periodic table. This technique provides a comprehensive elemental compositional characterization and it has the ability to measure concentration of elements at very low levels. Even concentration of 1-10 mg/l of analyte element can be detected. [35]

In inductively coupled plasma-mass spectrometry the analyte elements are ionized in inductive coupled plasma (ICP) and the ions are separated and measured by a mass spectrometer [35].
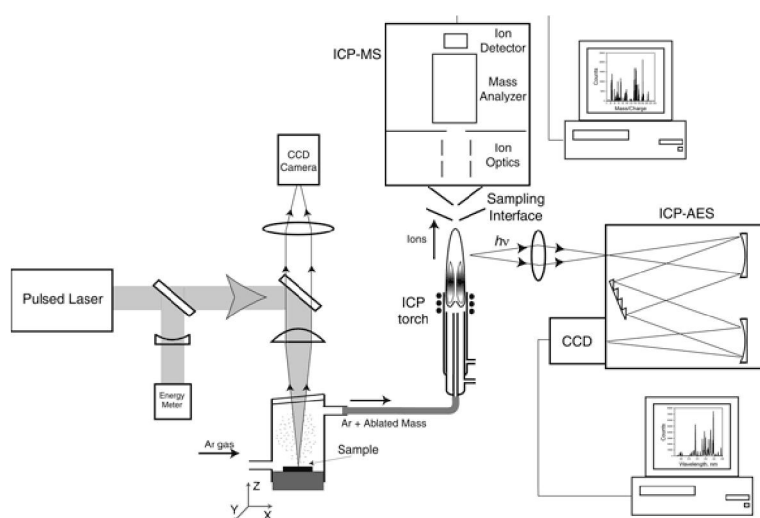
Figure 4. Plasma mass spectrometry. [36]

2.5.5 Anodic stripping voltammetry

Anodic stripping voltammetry is based on the tendency of silver ions to form insoluble salts with some anions. The first step in the process is to prepare a pre-concentration of the analyte onto the electrode followed by the successive stripping of the cumulate compound towards the solution [37]. A negative potential is applied to the electrode discharging the cations as metallic atoms into the electrode. The technique is sensitive and concentrations below 1 μg/l can be achieved [37].

2.5.6 Cyclic voltammetry

Cyclic voltammetry is a widely used method for qualitative determination of electrochemical reactions. It has the ability to provide information on the kinetics of heterogeneous electron-transfer reactions and on thermodynamics of redox processes. [17]

The electrode potential of a stationary working electrode is ramped linearly first to a more negative potential and after that back to the starting voltage. A current peak is produced by the forward scan if an analyte is reduced through the range of potential scan. The current increases as the reduction potential of the analyte is reached and then falls off as the concentration of the analyte is decreased close to the electrode. The dissolution behaviour of silver can be investigated with cyclic voltammetry. [17]

3 Major factors to silver dissolution

Several factors including pH, temperature and composition of the solution have effect on dissolution of silver. Those factors are described in this chapter.

3.1 pH

The metallic silver dissolution is affected by pH of the solution. The effect of pH has been investigated by studying the solubility of silver in ammonia solution with the presence of hydrogen peroxide or some halogen based oxidants. It was found that Iodine was superior as oxidant compared to bromine and hydrogen-peroxide. The dissolution behaviour of silver as a function of pH achieved in the study is similar to the metal complex speciation predicted from thermodynamic analyses. The values of pH in which the dissolution was greatest are found to be between 9 and 10. [38]

The effect of pH to the bacterial growth on silver nanoparticle has been studied by Fabrega et al [15]. They investigated the impact of silver particles on certain bacteria at pH values between 6 and 9 [15]. The size, aggregation, morphology, surface properties and dissolution of silver nanoparticles were characterized under all conditions. The physicochemical properties of silver nanoparticles were characterized at concentrations between 0 and 19 μM at pH values of 6, 7.5 and 9. The solubility of Ag nanoparticles was determined and it was found that the concentration of dissolved silver was low at all pH values. However the concentration increased slightly as pH value increased and was the highest at pH value of 9. The concentration of dissolved silver (Ag^+) increased also as the concentration of the silver nanoparticles increased. In addition to these results, the study showed also that the toxicity of solution increased at higher concentrations of silver nanoparticles and higher values of pH. [15]

3.2 Temperature

The increase in temperature improves the solubility of silver compounds. Figure 5 shows the correlation between temperature and the solubility of some silver compounds. It can be noted that the effect of temperature to the solubility is higher with compounds of lower solubility compared to the compounds that have higher solubility.
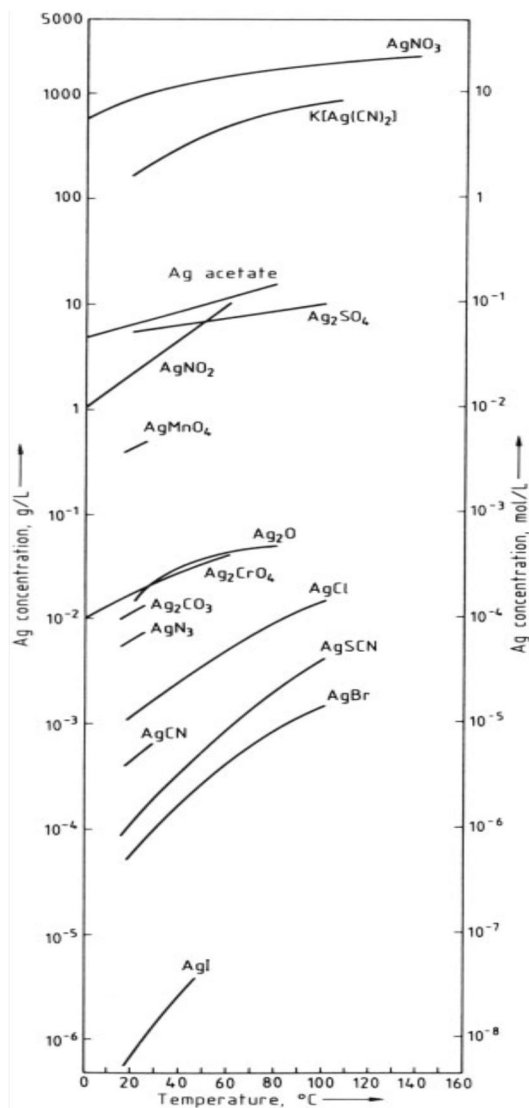
Figure 5. Solubility of silver compounds as a function of temperature. [12]

3.3 Halides

As the destruction of bacteria requires silver ions, the most effective form of antibacterial silver is soluble silver. Halide ions act as precipitating agents disturbing silver's effect on bacteria.

3.3.1 Effect of halides on toxicity of silver

For chloride ions, there are three levels of effects. Low chloride concentration does not affect the toxicity of silver. Moderate levels of chloride react with silver forming

precipitated AgCl which lowers the bioactivity. However higher levels of chloride and thus higher level of Cl$^-$ ions causes the formation of 'bioavailable' AgCl$_2^-$ ions increasing the Ag(I) sensitivity of bacteria. [2]

Br$^-$ affects in a similar way as Cl$^-$ but it functions at lower concentrations as a result of lower solubility of AgBr compared to AgCl [2].

Iodine behaves differently compared to chloride and bromide as it basically forms non-bioavailable AgI precipitate with silver [2].

3.3.2 Effect of halides on dissolution behaviour of silver

Halides have an effect also on silver dissolution behaviour. The formation of AgCl film is a result of the oxidation of silver in chloride solution [5].

$$Ag + Cl^- \rightarrow AgCl + e^- \tag{3}$$

First an AgCl monolayer is formed on a silver surface by adsorption-desorption method [37]. Multi-layer AgCl nucleates and grows at a certain potential more positive than the AgCl reversible potential. At a longer exposure time a thick AgCl film is resulted. [3]

It is essential for continuous corrosion that ions transport through the corrosion product layer. As the AgCl layer formation occurs there must be a dominant charge carrier inside the AgCl layer. This dominant charge carrier is still controversial. It has been suggested that silver (I) ion is the dominant charge carrier through AgCl layer inside the micro channels [3]. Another suggestion is that both silver and chloride ions contribute to the process [3]. Ha and Payer [3] demonstrated that formation of AgCl occurs at both the top and bottom of the silver chloride layer. This implies that the silver ions must transport through the AgCl layer to the electrolyte and chloride ions transport from the electrolyte to the substrate. The formation and thickening of the AgCl layer decreases the corrosion rate as the transportation of ions is decelerated. [5]

It has been found that in chloride electrolyte on a silver electrode surface the first monolayer that is formed is most likely a mixture of AgCl and AgOH. The second monolayer is AgCl and it occurs at higher potentials [17]. Horanyi et al. [17] studied anion adsorption at silver electrode and the conclusion was that in anodic dissolution the onset is preceded by notable adsorption of the anion. In addition there are other

studies showing that a monolayer of Cl⁻ - ions is absorbed before the electrochemical formation of bulk AgCl occurs. [17,39]

3.4 Ammonia

Ammonia reacts with silver forming a complex Ag[NH$_3$]$_2$$^+$. Accordingly, ammonia binds silver ions in the solution reducing the amount of silver ions and thus the bioactivity of silver. Silver dissolution is greatly affected by the ammonia concentration. [37]

The following reactions occur [39]:

$$Ag^+ + NH_3 \rightarrow Ag(NH_3)^+ \tag{4}$$

$$Ag(NH_3)^+ + NH_3 \rightarrow Ag(NH_3)_2^+ \tag{5}$$

3.5 Other factors

In addition to the above-mentioned there are also some other factors affecting the silver dissolution. Silver dissolves in cyanide solutions forming complex with cyanide ion. This complex is poorly soluble in water and reduces the bioactivity of silver.

The anodic dissolution of silver in cyanide containing solution proceeds as equation 6 represents [19].

$$Ag + 2CN^- \rightarrow Ag(CN)_2^- + e^- \tag{6}$$

Silver reacts also with gaseous and aqueous hydrogen sulphide forming Ag$_2$S. Silver sulphide is insoluble in water.

Silver forms a highly soluble compound silver(I) fluoride (AgF) in the reaction of silver(I) oxide or silver carbonate with hydrofluoric acid [12]. It is also formed as precipitated silver powder is dissolved in hydrofluoric acid and hydrogen peroxide. [12]

Nitric acid is often added to silver particles containing solution to stabilize the solution. Silver dissolves in concentrated nitric acid in the presence of oxygen [12,19].

$$3\mathrm{A}g + \mathrm{NO}_3^- + 4H^+ \leftrightarrow 3Ag^+ + NO + 2H_2O \tag{7}$$

Silver does not dissolve in sulphuric acid in normal conditions but it reacts with hot concentrated sulphuric acid forming fairly soluble silver sulphate [12]. The soluble and insoluble compounds of silver are listed in Table 1 [12].

Table 1. The solubility of silver compounds in water.

Highly soluble compounds	Moderately soluble compounds	Poorly soluble/ Insoluble compounds
$AgNO_3$	Ag_2SO_4	AgI
$K[Ag(CN)_2]$	Ag acetate	$AgBr$
Ag_2SO_4	$AgNO_2$	$AgCN$
Ag acetate	$AgMnO_4$	$AgCl$
AgF	$AgBF_4$	Ag_2O
$AgClO_4$	$AgClO_3$	Ag_2CrO_4
		Ag_2CO_3
		Ag_2S
		$AgNCO$
		$AgSCN$
		$[Ag(NH_3)_2]+$

Figure 6 represents the effect of temperature and the concentration of sulphuric acid and nitric acid to the rate of the silver dissolution. The solubility of different silver compounds in aqueous electrolytes is illustrated in the Figure 7.
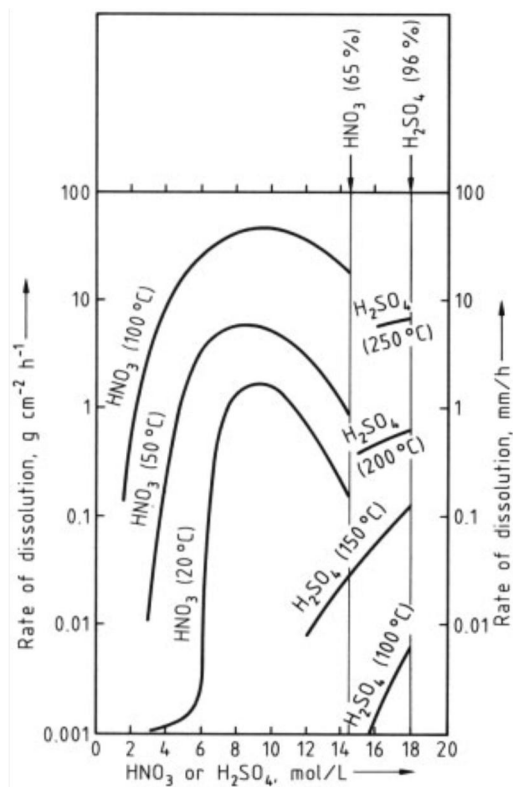
Figure 6. Dissolution rate of silver in sulphuric acid and nitric acid as a function of temperature and concentration. [12]
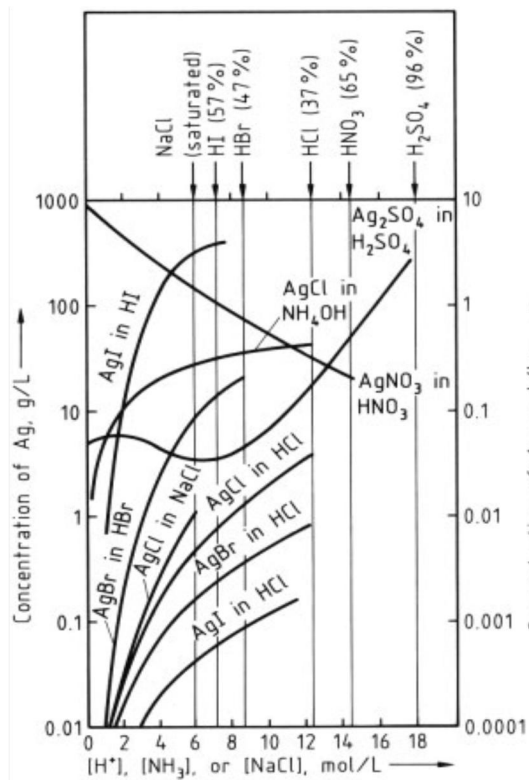

*Figure 7. Solubility of silver compounds at 20 °C in aqueous electrolytes. [12]*4 New

4 Antibacterial silver applications

Properties of a surface such as superhydrophobicity and photoactivity can be exploited in antibacterial applications. This chapter describes the mechanism of superhydrophobicity and photoactivity.

4.1 Silver particles on superhydrophobic surface

Superhydrophobicity can reduce the adhesion of bacteria to the surface. Combined with antibacterial activity of silver, superhydrophobic surface could form an efficient coating for many applications requiring purity and bacteria free environment. This chapter introduces

4.1.1 Superhydrophobic surfaces

Superhydrophobic surfaces are surfaces with water contact angle above 150º and rolling angle less than 10º. Superhydrophobic surfaces can be fabricated by combining surfaces of low surface energy with appropriate surface roughness. The principle of self-cleaning hydrophobic surface is presented in Figure 8. There are many surfaces in nature that are hydrophobic such as wings of butterflies and some plants like cabbage and the leaves of the lotus plant (Figure 9).

There are two kinds of structures of superhydrophobic surfaces in the nature. One is unitary micro-line structure and the other is hierarchial micro- and nanostructure. This is very important knowledge in the development of synthetic method to produce superhydrophobic surfaces. [40]

Superhydrophobic surfaces have a low rolling angle and the water droplet carries away surface contaminations when rolling over it. This is called the self-cleaning effect. [7,8]

Figure 8. Superhydrophobic surface and the self-cleaning effect. [41]

In addition to the self-cleaning property, superhydrophobic surface has been shown to have anti-bacterial property [42]. The contact area between the surface and the bacteria is reduced and the bacteria cannot easily adhere to the surface [43].

Superhydrophobic surfaces provide an opportunity for the development of many industrial products. For example current conduction and adherence of snow are inhibited on a superhydrophobic surface.

Superhydrophobic surfaces are profited in several applications such as corrosion-resistant, self-cleaning anti-icing surfaces, liquid separation, biomedical devices and in water bearing pipes reducing turbulent flow. [40]

Figure 9. Water droplets on lotus leaf. [44,45]

4.1.2 Processing of a superhydrophobic surface

The hydrophobicity enhances when the surface energy lowers. The wettability and thus hydrophobicity of a solid surface is usually evaluated by the contact angle of the water and the surface.

The surface of a lotus leaf has protruding nubs of approximately 20-40 μm apart from each other. These nubs are covered with smaller scale wax crystalloids consisting of –C-H and -C-O bonds [40]. Artificial superhydrophobic surfaces are fabricated by two different approaches: creating hierarchial micro- and nanostructures on hydrophobic surfaces or chemically modifying a surface structure from a low surface free energy material. [40]
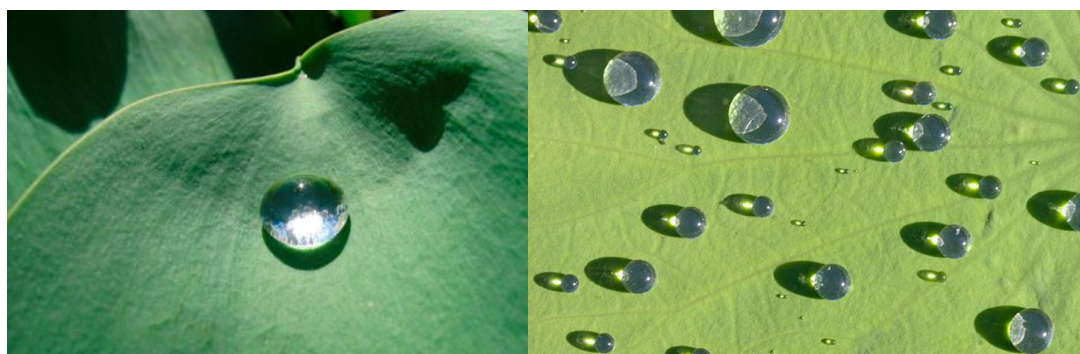
Lower surface energy can be achieved with silyl compounds or fluorocarbons (CF$_3$ groups). However these are not necessary needed as can be seen in the nature. There have been several attempts to produce artificial superhydrophobic surfaces without compounds that lower the surface energy but none of them have yet been a success. [40]

4.1.3 Methods to produce superhydrophobic surfaces

There are several methods for attaining surfaces that exhibit superhydrophobicity including electrochemical deposition, phase separation, chemical vapour deposition (CVD), crystallization control, lithography, sol-gel process, wet chemical reaction and electrospinning. [40]

Many other properties have been added to water repellency such as color change, transparency, flexibility, electrowetting and breathability. Combining of these properties can lead to more advanced materials for demanding applications. [40]

4.1.4 Advantages and applications of silver doped superhydrophobic surfaces

Superhydrophobic surface doped with silver particles could find potential applications in packaging and textile industry. Antibacterial and water repellent materials could be profited for example in hospital environment. The combination of these two properties has already been tested by adding silver micro-size particles on cotton textile surface. The silver particles containing surface was modified with

octyltriethoxysilane to obtain superhydrophobic surface. The modified cotton textile showed antibacterial activity against both gram-positive and gram-negative bacteria. [46]

4.2 Silver particles on titania surface

Titanium dioxide TiO_2 is a stable material with high photocatalytic activity. Photocatalysis can be used to destroy micro-organisms on the photocatalytic surface. Addition of silver particles can increase the photocatalytic activity of TiO_2 and thus the antibacterial activity of the surface. This chapter describes photocatalysis and effect of silver addition to titanium dioxide photocatalysis.

4.2.1 Photocatalysis

Photocatalysis is a process in which light of certain energy causes electrons to move from the valence band of a semiconducting material to the conduction band. The energy of the photon must be larger than the band gap of the semiconducting material. The electron and the hole that is created to the valence band can move to the surface of the material and react there with molecules producing radicals that have high oxidizing and reducing power. Organic molecules including micro-organisms can be degraded to carbon dioxide and water. Mechanism of photocatalysis is presented in Figure 10.

Photocatalysis is used for example for self-cleaning, antibacterial and anti-soiling surfaces, air purification and water treatment. [47] Photocatalytic materials possess also superhydrophilic property when exposed to irradiation. Superhydrophilic surface has water contact angle close to 0^0 which leads to formation of a water film on the surface. [48]

The most studied photocatalysts are titanium dioxide (TiO_2) and zinc oxide (ZnO) because of their photochemical stability, high photosensitivity and non-toxic nature [49]. TiO_2 is physically and chemically stable and it has high photocatalytic activity and high oxidative power. Titanium dioxide has a wide band gap (3,2 eV for anatase and 3,0 eV for rutile) which limits its use in visible light. Anatase is the most photocatalytically active polymorph of titania in most reactions [5]. The bandgap of zinc oxide is 3,37 eV [50]. Other photocatalytic materials are for example WO_3, ZnS and carbon nanotubes that have been prepared using a specific heat treatment [49,51,52].

In a semi-conducting photocatalyst the rate of recombination of photoexcited electrons and holes influences the photocatalytic efficiency. During the photocatalytic process the valence electrons excitate to the conduction band leading in the formation of holes in their previous position in the valence band. The lifetime of the electrons and holes is short and in the absence of suitable scavengers recombination of the electrons and holes occurs. [5]

Fresh titanium dioxide possesses a strong germicidal activity with and without UV-irradiation. The effect is stronger with gram-negative cells compared to gram-positive ones. The germicidal effect gradually decreases as the TiO_2 is exposed to air as a consequence of decrease in the wetting property. The germicidal activity of fresh TiO_2 has been found to be even stronger than that of TiO_2/UVA or Ag^+. These results indicate that a long lasting germicidal activity could be achieved if the wetting property is maintained. [53]

Figure 10. Basic mechanism of photocatalysis.

4.2.2 Photoactivity of silver metal nanoparticles

Some metal nanoparticles such as silver and gold show photoactivity when they are irradiated with UV-visible radiation. This is characteristic to only nanoparticles whereas noble metals in bulk are photoactive only to a small extent [54]. Metal nanoclusters may have three different mode of action including direct excitation of metal nanoparticles, indirect excitation achieved via dye molecule or surface bound

fluorophore and assisting photocatalytic process. The latter occurs by promotion of interfacial charge transfer in semiconductor-metal nanocomposites [46]. Metal and semiconductor nanoclusters have some similarities in their physical properties such as optical transparency and they both act as dipoles. Metal nanoclusters have close lying conduction and valence band whereas the bandgap of semiconductor nanoclusters is well-defined. [54,55]

4.2.3 Effect of silver doping on titanium dioxide photoactivity

The possibility of increasing visible light activity to TiO_2 by doping with metal ions has been researched in numerous studies. [5,56,57] Noble metals such as silver seem to have the ability to reduce the recombination reaction. Excited electrons from TiO_2 can be trapped by silver and thus photogenerated holes react with water molecules creating OH^- radicals. These radicals react with organic molecules resulting to degradation reaction of organic species. Silver particles create also a local electric field which may result to electron excitation. [5,58,59]

There are many studies showing the increase of photocatalytic activity when silver is added on the surface. However, the exact mechanism is unknown and some contradictory results are reported. These results showing decrease of photocatalytic activity of silver doped titania may result from metal content and the dispersion of metal or the preparation method of silver modified titania [5,6]. Even though silver seems to have a positive effect on photocatalytic activity it should be noticed that reduction of metal ions may occur on the surface of the photocatalyst. If silver ions are reduced to metallic silver the antibacterial activity decreases.

The fabrication method should be elaborated when titanium dioxide coatings with silver nanoparticles are produced. Some methods such as chemical vapour deposition (CVD), sol-gel and aerosol methods have been successful. [59]

4.2.4 Influence of Ag doping on the TiO_2 grain size and phase transformation

It seems that the addition of silver affects the grain size of titania. He et Al. [59] produced silver nanoparticles doped titania particles by sol-gel method. In that study it was found that silver addition decreased the produced grain size. Apparently large silver ions migrated on the produced surface and interfered the anatase grain growth. [59]

Doped titania particles have been produced from chloride precursor and it has been found out that the doped element inhibits the formation of rutile. Silver doping of titania has also decreasing effect on the primary size of the particles and the aggregate size. Ag doping could also have an effect on the titania particle shape [59]. H. Keskinen et al. [59] produced silver-titania deposits consisting of nanoparticles by liquid flame spray. They showed that the agglomerate size and the primary particle size decreased when the amount of silver increased.

It has also been shown that the phase transformation temperature of the doped titania powder decreases as the amount of doped silver increased. In that study pure and silver doped TiO_2 powders prepared by the sol-gel process were calcined at different temperatures. Both powders were well crystallized at 500 ℃. Contrary to the silver doped powder, the phase transformation from anatase to rutile for the pure powder did not occur below 750 ℃. [56]

4.2.5 Applications and advantages of silver doped titanium dioxide

As the addition of silver particles on titanium dioxide increases the antimicrobial activity in most cases, Ag/TiO_2 composite is suitable for many applications requiring enhanced antibacteriality. There are already some applications using Ag/TiO_2 coatings such as textiles and orthopaedic implants. [21,60]

The antimicrobial activity of textile with silver doped silica spheres was tested and the results indicated that textiles with silver located on silica spheres demonstrated antibacterial effect on both gram positive and gram negative bacteria. On the contrary the textiles that were doped with colloidal commercial silver did not show antibacterial activity. [21]

Due to the stability to cleaning and use, excellent antimicrobial response and robustness of Ag- doped titania coating it is a potential coating material for hard surfaces in hospital environment [61].

Results of a study by Guo et al. indicate that Ag/TiO_2 nanotubes could cause photo-degradation or transformation of some pollutants to detectable states in analytical means [62].

4.3 Introducing of TiO_2 photocatalyst on superhydrophobic membrane

Superhydrophobic surfaces can be profited for example when the attachment of water or snow on the surface must be prevented. However, there is an increasing amount of pollutants in the environment and even though superhydrophobic surfaces have the self-cleaning property it may be covered by dust in time. That would lead to the fading of the superhydrophobic property. In some cases this problem has been solved by coating of titanium dioxide on superhydrophobic membrane. Good results have been achieved in some cases when porous Teflon has been used as superhydrophobic coating material [63].

With a small amount of TiO_2 the water contact angle increased compared to the angle on surface without any TiO_2. However, as the amount of TiO_2 increased the water contact angle reduced at longer irradiation times. The use of small amount of TiO_2 led to the purification of the surface and it recovered the superhydrophobic property of the surface [63].

Combination of photocatalytic and superhydrophobic properties could be used in many applications demanding water repellency and sanitation.

5 Experimental

In this chapter, methods, chemicals and equipments used in the process of preparing superhydrophobic silver containing surfaces and measuring the solubility of silver from the suprhydrophobic surfaces and commercial silver particles are described. The process to produce superhydrophobic aluminum oxide based coatings used in this thesis is formerly described in the Thesis for the degree of Doctor of Technology: 'Nanocrystalline Functional Alumina and Boehmite Materials: Synthesis, Characterization and Applications' by Xiaoxue Zhang at Tampere University of Technology in 2009. A method to produce hydrophobic coating using titanium dioxide as a base material is also described in this chapter.

5.1 Methods to produce surfaces used in this thesis

There are different methods to produce superhydrophobic surfaces. In this thesis a process in which sol-gel method is used to form a ceramic coating with a flaky structure was employed. In this chapter the sol-gel method and the stability of different ceramic materials are introduced.

5.1.1 Sol-gel process

Sol-gel method is a simple technique to produce almost any oxide powders and coatingsIn the sol-gel technique, metal alkoxides are dissolved into an organic solvent to be followed by reactions with water. These reactions are hydrolysis and condensation. When controlled hydrolysis and condensation reactions are realized, the hydroxyl ion attaches to the metal atom of the alkoxide and disconnects the alkyl group of the compound. In the next step, hydrolyzed molecules undergo a condensationreaction between -OH groups liberating small molecule, which is normally alcohol or water. [64] Hydrolysis and water liberating condensation reaction are presented in equations 8 and 9.

$$M(OR)_x + xH_2O \rightarrow M(OH)_x + xROH \tag{8}$$

$$2M(OH)_x \rightarrow M_2O_x + xH_2O \tag{9}$$

Conventionally, sol-gel reactions are catalyzed by acids or bases and the final structure of the produced powder or coating depends on the post-treatment. Typical post-treatment is calcinations. [64]

5.1.2 Structure of superhydrophobic ceramic coatings

Hydrophobicity raises as surface-energy decreases and surface roughness increases. Flaky alumina structure can be fabricated relatively easily. Alumina is a stable material. The Gibbs energies of formation of different oxides are listed in Table 2. [65]

Table 2. Gibbs energy of formation for different oxides in descending order in the table.

Oxide	Gibbs energy of formation (kJ*mol^{-1}/metal atom at 25ºC)
ZrO_2	-1039,724
TiO_2	-889,406
SiO_2	-856,443
Al_2O_3	-791,138
CaO	-603,501
B_2O_3	-596,40
BeO	-573,209
MgO	-568,945
BaO	-520,382
CO_2	-394,389
Fe_2O_3	-371,76

The relative stabilities of three different alumina polymorphs are listed in Table 3. α - alumina is the most stable for of alumina followed by δ and γ a-alumina. [65]

Table 3. Relative stability of Al$_2$O$_3$ polymorphs (kJ/mol).

Temperature (K)	α-Alumina	δ-Alumina	γ-Alumina	κ-Alumina
298,15	-1582,275	-1572,974	-1563,850	-1569,663
1000	-1361,437	-1353,447	-1346,370	-1351,782
2000	-1034,096	-1031,160	-1028,802	-1030,989

High surface area can be achieved by increasing the surface roughness and lowering surface-energy with flaky structure. There are more than one way to achieve flaky alumina structure. The method used in this thesis is described in chapter 5.1.1.

5.2 Preparation of the surfaces

The preparation process of superhydrophobic alumina based surfaces is presented in this chapter. The addition of nanosized silver particles via Tollens process is also presented.

5.2.1 Preparation of the superhydrophobic surfaces

Superhydrophobic surfaces were prepared on AISI 316 2B austenitic stainless steel substrates. The size of a substrate was 75 mm x 25 mm. The plates were washed first with acetone, then wiped with nitric acid (HNO_3) and rinsed with water and finally wiped with acetone.

The flaky gamma-alumina layer was prepared by sol-gel method. 3,0 g aluminium-tri-sec-butoxide was added to 50,0 ml isopropanol (C_3H_7OH) and the solution was stirred for one hour at room temperature. 2 ml ethyl acetoacetate was introduced to the solution and the solution was again stirred for one hour. 1,0 ml de-ionized water was mixed with 5,0 ml isopropanol and the mixture was slowly added to the initial solution for the hydrolysis. The solution was stirred for another two hours and was then ready for coating.

Spin coating was used to obtain a thin uniform layer. The coating was carried out at 1500 rpm for 20 seconds on the cleaned steel plates.
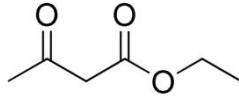
After the samples were dried at room temperature for couple of minutes they were heat-treated at 400 ºC for 15 minutes in air. After cooling down the samples were immersed into boiling water for 10 minutes. Aluminum hydroxide dissolved from the surface during boiling and a crystalline boehmite structure was formed. The samples were then heat-treated at 600 ºC for 30 minutes in air and boehmite transformed to γ-alumina but flaky morphology was restored.

A layer of FAS ((heptadecafluoro-1,1,2,2-tetrahydrodecyl)trimethoxysilane) was added on the alumina structure to obtain superhydrophobic surface. 1,0 ml FAS and 50,0 ml ethanol were stirred for 1 hour at room temperature and the alumina coated plates were immersed into the solution for 15 minutes. After the samples were dried at room temperature for 15 minutes they were heated at 180 ºC for one hour in air.

During the heat-treatment alumina layer formed bonds with FAS molecules in a condensation reaction.

All the chemicals used in the process are listed in Table 4. The compositions of the chemicals are also listed.

Table 4. Chemicals and their compositions used in surface preparing process.

Chemical	Purity	Chemical composition
Aluminum-tri-sec butoxide	97%	$C_{12}H_{27}AlO_3$
2-propanol (isopropanol)	99%	C_3H_7OH
Ethyl acetoacetate	98%	
De-ionized water		H_2O
FAS, (heptadecafluoro-1,1,2,2-tetrahydrodecyl)trimethoxysilane		$(CF_3(CF_2)_7CH_2CH_2Si(OCH_3)_3$
Ethanol BA	99%	C_2H_5OH

Another way to achieve smilar structure by more simple method is to use hydrolysis of AlN powder at 90 ^{o}C and deposit a boehmite coating on to a substrate. The structure is shown in Figure 11. After heat treatment in 500 ^{o}C the surface transforms to γ-alumina and at 900 ^{o}C to δ-alumina. A subsequent chemical modification of the coating with low-energy surface chemical produces superhydrophobic surface. [66]
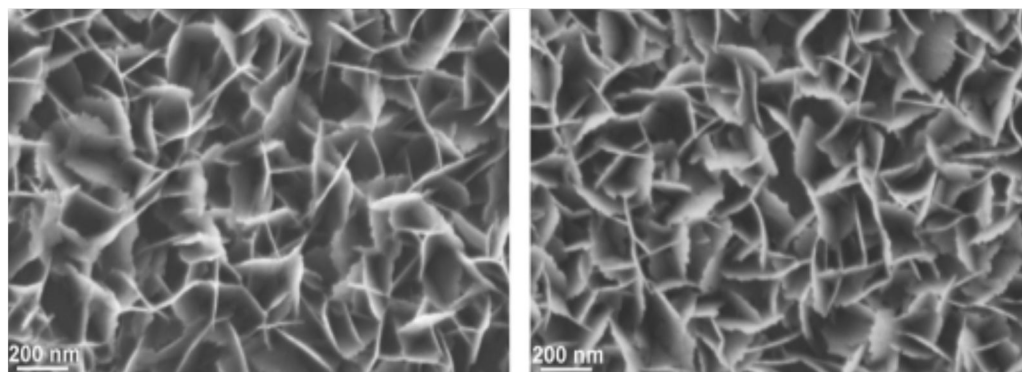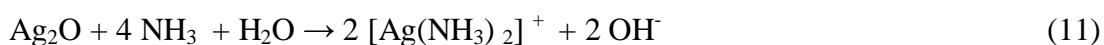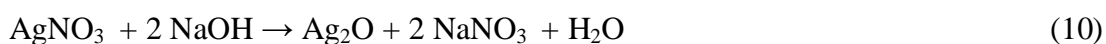
Figure 11. On the left SEM image of the precipitated boehmite coating and on the right the same coating after heat treatment at 900 ℃ for 1 hour. [66]

5.2.2 Addition of silver by Tollens process

Silver particles were added on the superhydrophobic surface by Tollens process. The silver was reduced on the alumina layer by the following procedure.

1% silver solution was prepared by adding 1,575 g silver nitrate ($AgNO_3$) to 100 ml de-ionized water. Then 5 ml sodium hydroxide (NaOH) was added and silver oxide (Ag_2O) precipitation formed. After that ammonia (NH_3) was added to solution until all Ag_2O had dissolved and silver had formed diamminesilver ($[Ag(NH_3)_2]^+$) complex ions. Finally a mixture of 10 ml de-ionized water and 1 g dissolved sucrose was added to the solution. Sucrose was used as a source of aldehyde (RCHO) to reduce diamminesilver to metallic form. The solution was positioned on the flaky γ-alumina layer by spin coating. The spin coating was carried out at 1500 rpm for 15 s. The samples were dried at 80 ℃ for one hour during which the reduction of silver occurred. Finally the top FAS layer was added on the surface as described previously. The reactions of the Tollens process are presented in equations 10, 11 and 12 [67].

$$AgNO_3 + 2\ NaOH \rightarrow Ag_2O + 2\ NaNO_3 + H_2O \qquad (10)$$

$$Ag_2O + 4\ NH_3 + H_2O \rightarrow 2\ [Ag(NH_3)_2]^+ + 2\ OH^- \qquad (11)$$

$$RCHO + 2\ [Ag(NH_3)_2]^+ + 2\ OH^- \rightarrow 2\ Ag + RCOOH + H_2O + 4\ NH_3 \qquad (12)$$

The chemicals and their chemical compositions are presented in Table 5.

Table 5. Chemicals and their compositions used in Tollens process.

Chemical	Chemical composition
Silver Nitrate	$AgNO_3$
Sodium hydroxide	$NaOH$
Ammonia	NH_3
Sucrose	$C_{12}H_{22}O_{11}$ (contains RCHO group)

5.2.3 Superhydrophobic antibacterial photocatalytic hybrid surfaces

A possibility to create a hybrid surface in which properties of an antibacterial metal, photoactivity of a photocatalytic material and superhydrophobicity would be combined was inspected. Hybrid surface would possibly have strong capability to prevent growth of microorganisms on the surface.

TiO_2 was chosen to be used as photocatalytic material because of the high photoactivity and stability of the material. Stainless steel plate was sandblasted to obtain high surface roughness. The sandblasted surface was coated with a titanium dioxide layer by sol-gel method. Then the plates were heat treated at 400 ⁰C for 1 hour in order to obtain anatase phase structure. The presence of anatase structure was confirmed by X-ray diffraction (XRD).

Silver was added on the surface by photocatalytic reduction of silver nitrate. Finally a layer of fluorosilane was added on the titanium dioxide coating. Superhydrophobic property was tested by water contact angle measurement and photocatalytic activity was tested by dye reduction test using resazurin ink.

The prepared titanium dioxide surface surface was rubbed with a cloth that was immersed in ethanol to investigate mechanical durability of the coating.

5.3 Characterization methods

Silver solubility from silver nanoparticles and superhydrophobic plates with silver was determined by using Atomic absorption spectroscopy (AAS). The superhydrophobic silver containing surfaces were characterized using field emission scanning electron microscopy (FESEM). The chemical resistance of the superhydrophobic surfaces was detected by measuring water contact angle and with field emission scanning electron microscopy after the coatings were exposed to different solutions. Methods used in microbiological tests and mechanical durability tests are also described in this chapter.

5.3.1 Characterization of superhydrophobic surfaces and commercial silver particles

The superhydrophobic surfaces with and without silver particles were characterized by field emission scanning electron microscope (FESEM). The presence of silver was detected with Energy dispersive spectrometry (EDS) -analyse. Commercial silver nanoparticles (particle size < 100 nm, Sigma Aldrich) were also characterized by field emission scanning electron microscopy (FESEM). The particle size and shape was determined from the images.

5.3.2 Solubility of silver from superhydrophobic surfaces and commercial particles

Silver release from commercial nanoparticles (particle size < 100 nm, Sigma Aldrich) was measured in de-ionized water and in acidic and alkaline environment. 20 μg of nanosized silver was added to 100 ml of the solution. The pH of the alkaline system was 12 and pH of the acidic system was 1. Nitric acid was used to adjust the pH 1 and ammonia was used to adjust the pH 12. The test was carried out for 72 days and the amount of dissolved silver was measured at certain time points with atomic absorption spectrophotometer. Before the measurement the solutions were centrifuged to separate silver particles from the measured solution. The solutions were kept in plastic enclosed containers in dark at room temperature.

Steel plates with superhydrophobic surface and with silver particles on the surface were immersed in three different solutions. The pH's of the solutions were adjusted with nitric acid and ammonia and were 1 and 12 as in the dissolution test for the

silver nanoparticles. The test was carried out for 72 days and silver concentration was determined at certain time points. Plastic enclosed containers were used to prevent silver adsorption to the container surface. The test was carried out at room temperature in the dark.

5.3.3 Total amount of silver on the surfaces

The total silver on the superhydrophobic surfaces was not precisely known as the addition of silver was made with spin coater by Tollens process. The total silver was determined by dissolving all the silver from the surface to a strong nitric acid solution and measuring the concentration (g/l) with atomic absorption spectrophotometer. As the total volume of the solution was known the mass of total silver could be determined like equation 13 presents.

$$mass\ (m) = concentration\ (c) * volume\ (V) \tag{13}$$

5.3.4 Chemical durability of superhydrophobic coatings

The chemical durability of silver containing superhydrophobic surfaces was investigated by measuring the water contact angle after exposure of the samples in acidic, alkaline and de-ionized water environment. The pH's of the acidic and alkaline environments were 1 and 12. The measurements were carried out after 2 and 4 weeks of exposure.

After the exposure of silver containing superhydrophobic coatings to solutions with different pH's the surfaces were investigated with FESEM to find out the chemical durability of coating in different environment.

5.3.5 Mechanical durability of superhydrophobic coatings

Mechanical durability of superhydrophobic coatings was tested by a nanoscratch test. Testing was performed in Manchester Metropolitan University by Professor Peter Kelly's group. Superhydrophobic surface was also rubbed with a cloth and water contact angle was measured before and after the rubbing. The change in the morphology after rubbing was detected using scanning electron microscopy.

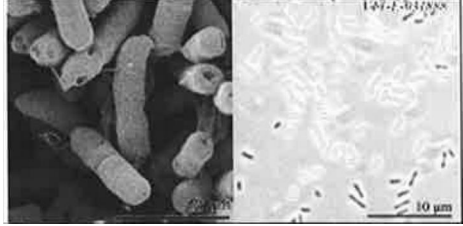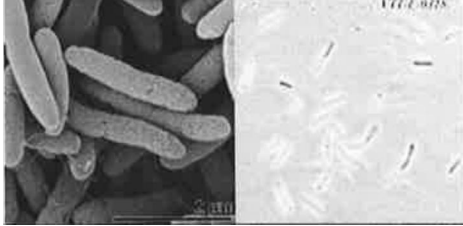## 5.3.6 Microbiological tests of superhydrophobic surfaces with and without silver

Superhydrophobic surfaces with and without added silver were tested by Mari Raulio at Technical Research Centre of Finland to investigate the efficiency of antimicrobial and antiadhesive surfaces. Bacterial attachment on surfaces was evaluated by inoculation of the surface by different methods (immersion, spray) and growth of cells on biofilm and investigating cells on the surfaces. Methods that were used for the detection were epifluorescence microscopy, sonication (enables subsequent colony counts), agar overlay (can enable counting of subsequent colony formation), atomic force microscopy (AFM) and videometer imaging (visual measurement of the microbe coverage). The microbial strains used in this study are listed in Table 6. Table 6 shows also the temperature at which bacterial strains were grown as well as the growing time. [68]

Samples were placed in the petri plates and they were covered with a lid and then incubated under shaking (40 rpm). The incubation was performed for 18 hours at 25ºC and biofilms that were formed on the samples were rinsed twice with sterile de-ionized water and then stained with nucleic acid selective fluorescent cationic dye (Acridine orange) or Live/dead-staining kit (contains two dyes, one for membrane permeable SYTO 9 labels and one for membrane impermeable propidium iodide labels) for 10 minutes at the dark. Samples were rinsed with de-ionized water and then left to air-dry. Samples were examined with epifluorescence microscope and the images were analysed with CellC software. Bacterial counts (bacteria cm^{-2}) and coverage area (%) of the biofilm were determined with CellC software. [68]

Antibacterial and antiadhesive properties were examined also with a method based on cultivation. The samples were placed on a petri plate and bacterial species were introduced to the sampled as described above and biofilm was formed and then rinsed. The bacteria adhered were removed by ultrasonication and vortexing. The solution with bacteria was deluted and cultivated to determine the amount of viable bacteria. [68]

Videometer imaging was used for measuring the microbial coverage of the samples. 20 spectral bands (360-1050 nm) were imaged with and without acridine orange staining. [68]

Table 6. Bacterial strains used in microbiological tests.

Strain	Cultivation	Description	Image (SEM, phase contrast)
Lactobacillus paracasei	30ºC, anaerobic	Gram-positive	[68]
Serratia marcescens	30ºC, aerobic	Gram-negative	[68]
Pseudomonas fluorescens	30ºC, aerobic	Gram-negative	[68]

5.3.7 Resazurin ink test

Resazurin ($C_{12}H_7NO_4$) is a dye that can be used as an oxidation-reduction indicator to determine photoactivity of a surface. Resazurin is dark blue in color. When resazurin is reduced it turns to pink resofurin. [69]

In this work 3 g of 1,5 wt% aqueous hydroxyethyl cellulose solution (Sigma Aldrich) and 0,3 g of glycerol (Sigma Aldrich) were mixed. Then 4 mg of resazurin dye (Sigma Aldrich) was added. The solution was stirred for 30 minutes and a thin layer of the solution was then spread on the surface. After that the surface was irradiated with UV-light (100 W/m^2) for 2 minutes. [69]

6 Results

In this chapter the results of the silver dissolution measurements and the images of superhydrophobic surfaces before and after the

6.1 Characterization of silver doped superhydrophobic surfaces

Surfaces were characterized by taking images using scanning electron microscopy. Figures 12 a - c present silver particles on superhydrophobic surface at different magnifications. The size of the particles is approximately 200 - 300 nm.
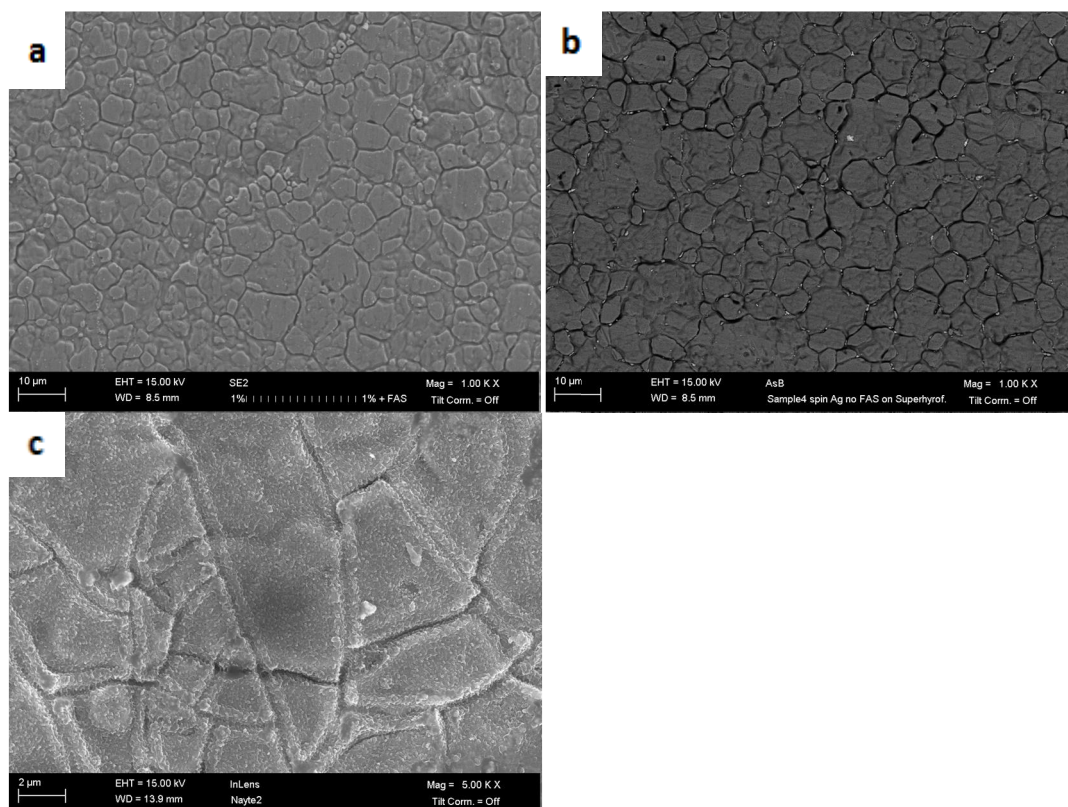

Figure 12 a - c. Scanning electron microscopy images of silver containing superhydrophobic surfaces at different magnifications.

Low magnifications show the structure of austenitic stainless steel but the topography of the flaky alumina surface cannot be seen. Backscattered electron (BSE) image (Figure 12 b) shows silver particles as white dots. Silver particles are

settled evenly over the surface. Larger magnification (Figure 12 c) reveals the topography of the surface.

Figures 13 a - d show higher magnifications of superhydrophobic surface with silver particles. The particle size of round shaped silver particles is approximately 200 - 300 nm. The size of the flakes of the alumina-FAS layer is in tens of nanometre scale. The shape of a flake is round shaped when the sample is coated with carbon whereas the shape of a flake without carbon coating is sharper.
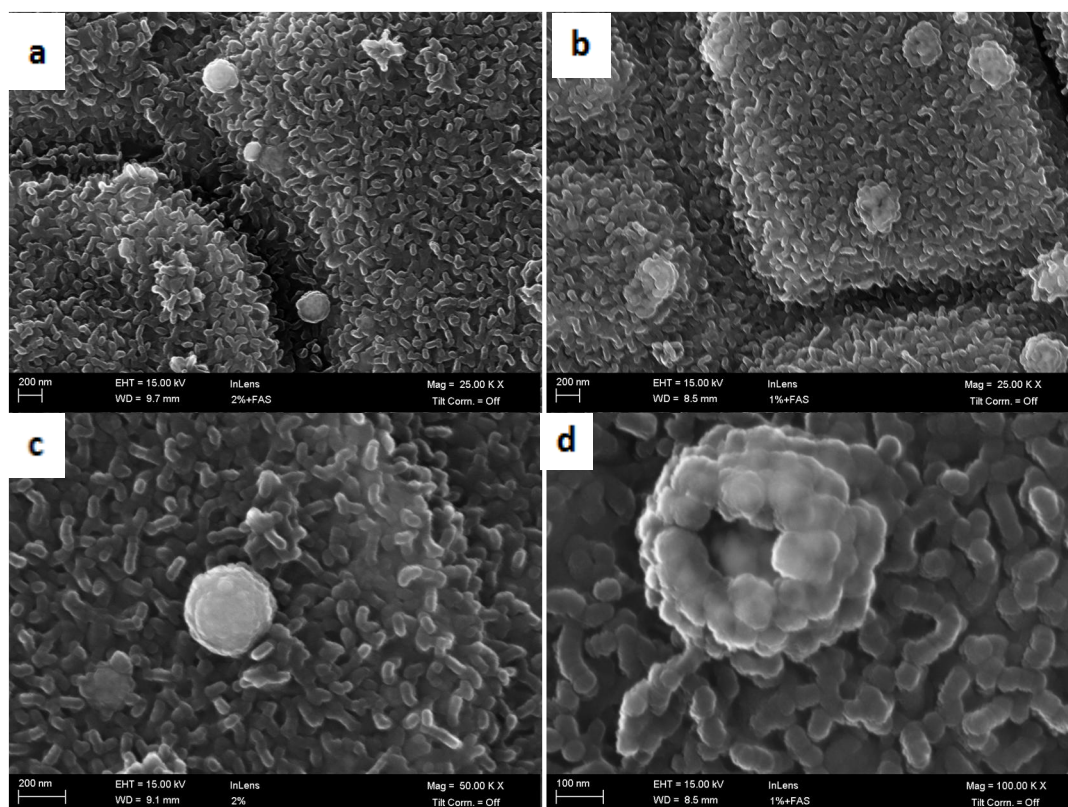

Figure 13 a - d. Silver particles on superhydrophobic silver doped surface with carbon coating at different magnifications.

Energy dispersive X-Ray analysis (EDS) was used to verify the chemical composition of the particles on the surface. The results of the spot analyses are shown in Tables 7 - 9. Figures 14 - 16 show the spots that the analyses were performed from. The results prove that round bright coloured particles on the surface are silver.

Table 7. EDS analysis of silver containing superhydrophobic surface.

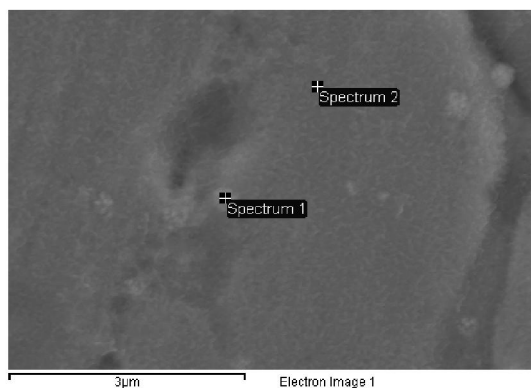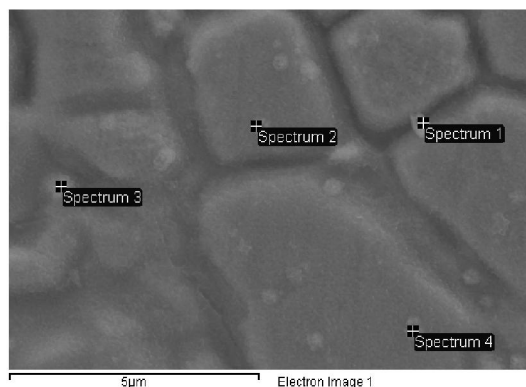| Spectrum	Ag (%)
Spectrum 1	15,24
Spectrum 2	-0,16

Figure 14. EDS analysis of silver containing superhydrophobic surface.

Table 8. EDS analysis of silver containing superhydrophobic surface.

Spectrum	Ag (%)
Spectrum 1	0,17
Spectrum 2	3,27
Spectrum 3	0,71
Spectrum 4	1,73

Figure 15. EDS analysis of silver containing superhydrophobic surface.

Table 9. EDS analysis of silver containing superhydrophobic surface.

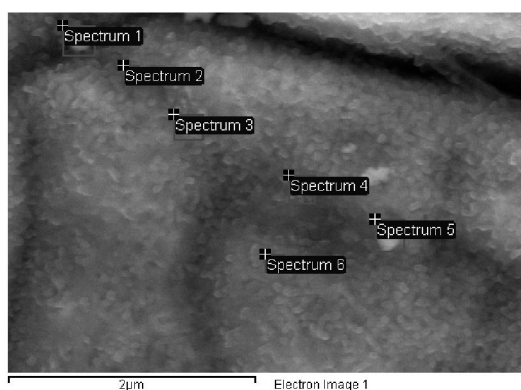| Spectrum	Ag (%)
Spectrum 1	10,73
Spectrum 2	5,28
Spectrum 3	6,48
Spectrum 4	5,45
Spectrum 5	7,23
Spectrum 6	1

Figure 16. EDS Analysis on silver containing superhydrophobic surface.

6.2 Characterization of commercial silver particles

Silver particles were characterized by taking images with scanning electron microscope (SEM). It can be seen from the Figure 17 a - e that nanoparticles have agglomerated to larger particles. With the images of high magnifications the size of the small particles can be evaluated to be approximately 100 nm.
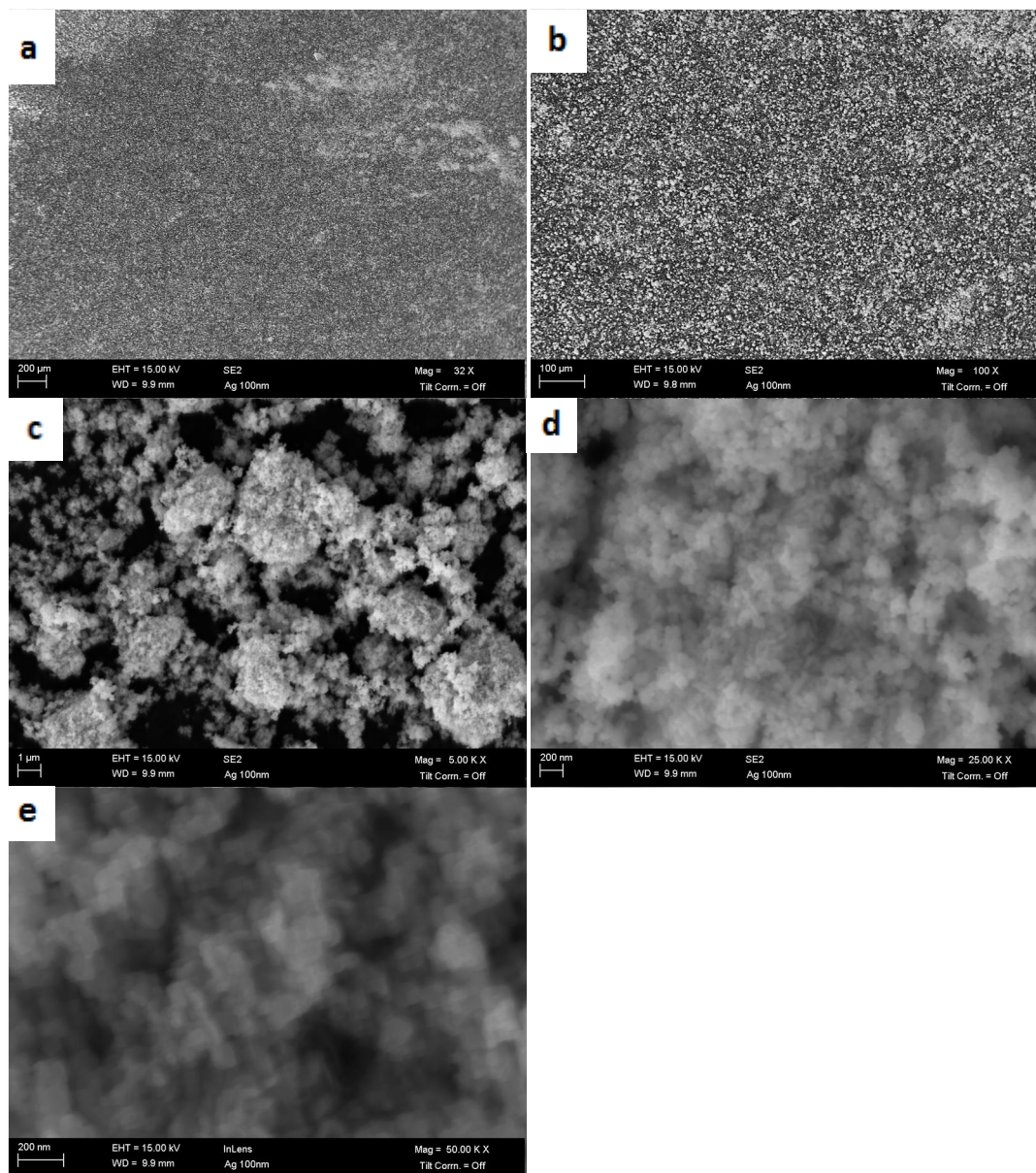
Figure 17 a - e. Scanning electron microscopy images of silver nanoparticles at different magnifications.

6.3 Solubility of silver from particles

Silver dissolves from commercial nanosized silver particles (< 100 nm) in nitric acid, de-ionized water and ammonia containing environment. The rate of dissolution is highest in ammonia solution and second highest in nitric acid. In water the dissolution is nearly linear whereas in ammonia nitric acid the dissolution rate increases fast and then stabilizes. Figure 18 shows silver ion concentration in different solutions as a function of time. Nitric acid can be used to keep ionic silver in the solution as it prevents the formation of unwanted precipitations and adsorption of the silver on the container. In de-ionized water it is possible that some of the dissolved silver has been adsorbed on the container.
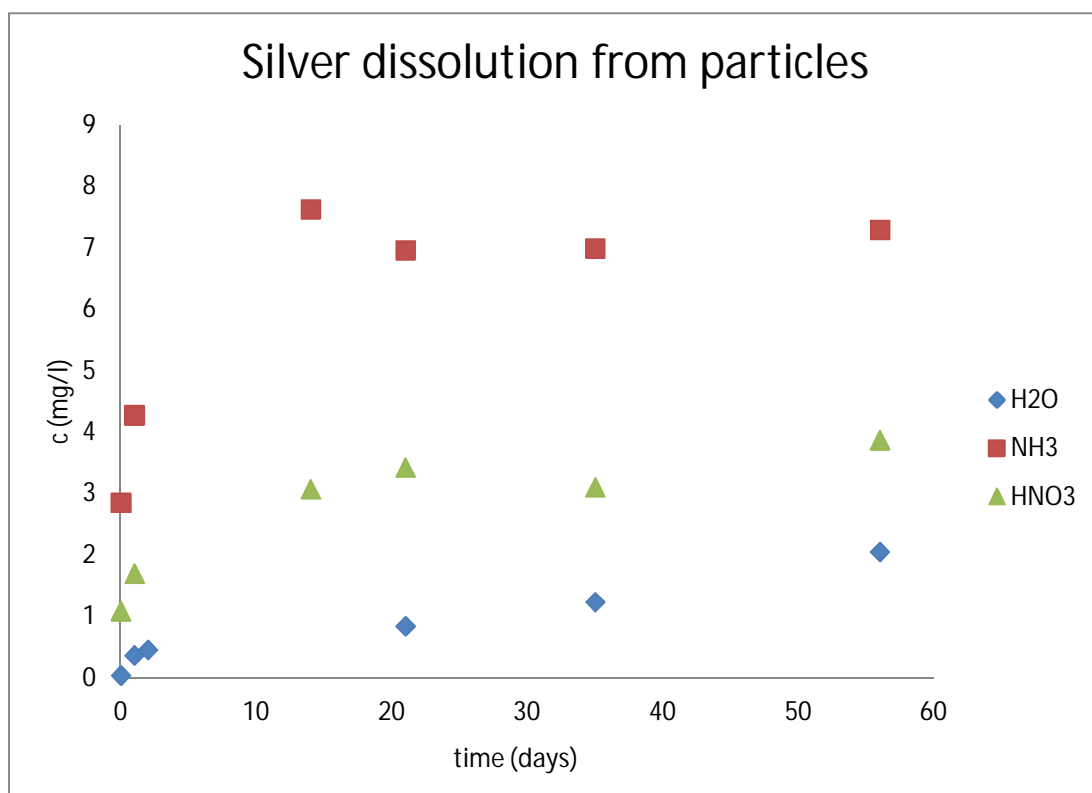

Figure 18. Silver ion concentration in water, ammonia and nitric acid solution as a function of time.

6.4 Solubility of silver from superhydrophobic coatings

Silver dissolves in all the tested solutions from superhydrophobic surfaces that contained silver. Interestingly dissolution rate was highest in nitric acid and only second highest in ammonia. This may result from the poor durability of the coating in nitric acid. The initial amount of silver might also have been slightly higher in the sample that was exposed to nitric acid compared to the one exposed to ammonia due to the silver plating system. Dissolution occurred also in de-ionized water but dissolution rate was notably lower than in acidic and alkaline media. In Figure 19 concentration of dissolved silver is presented as a function of exposure time.
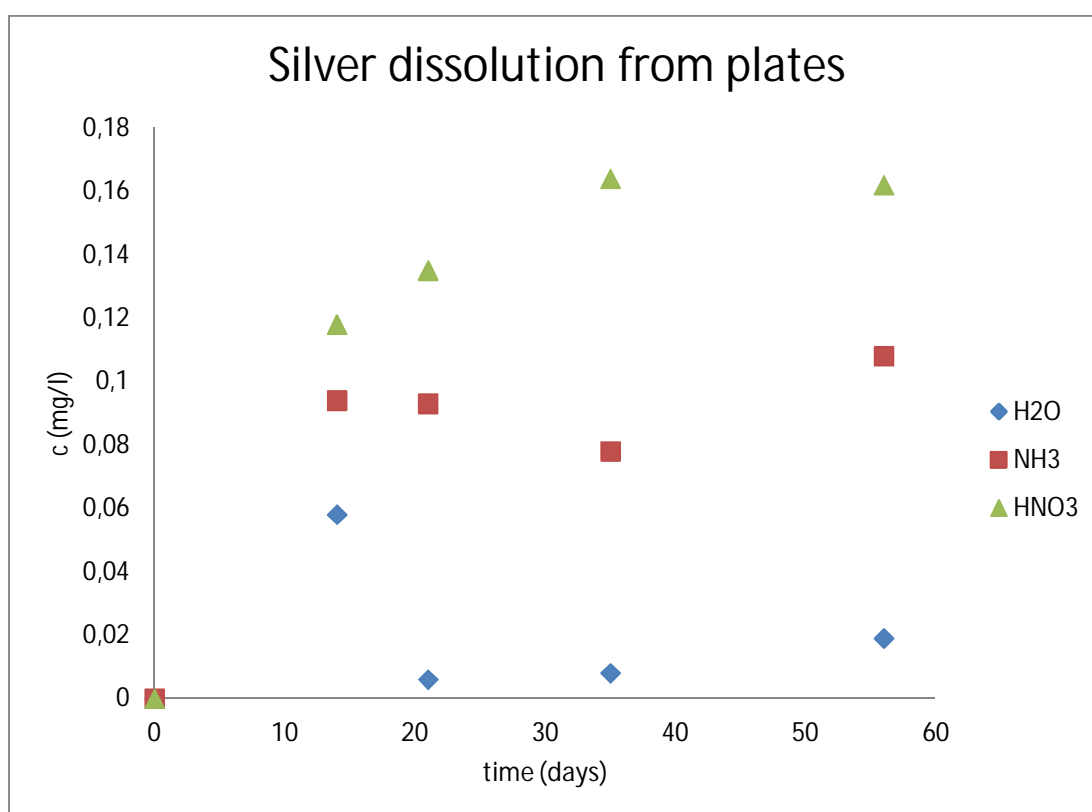

Figure 19. Silver ion concentration in water, ammonia and nitric acid solution as a function of time.

Dissolution of silver from the superhydrophobic coating with addition of silver in ethanol occurred during 56 days. Silver concentration of the solution was 0,032 mg/l whereas in de-ionized water silver concentration of solution after 56 days was 0,019 mg/l. Figure 20 presents the concentrations of dissolved silver after 56 days of exposure in de-ionized water, nitric acid, ammonia and ethanol.
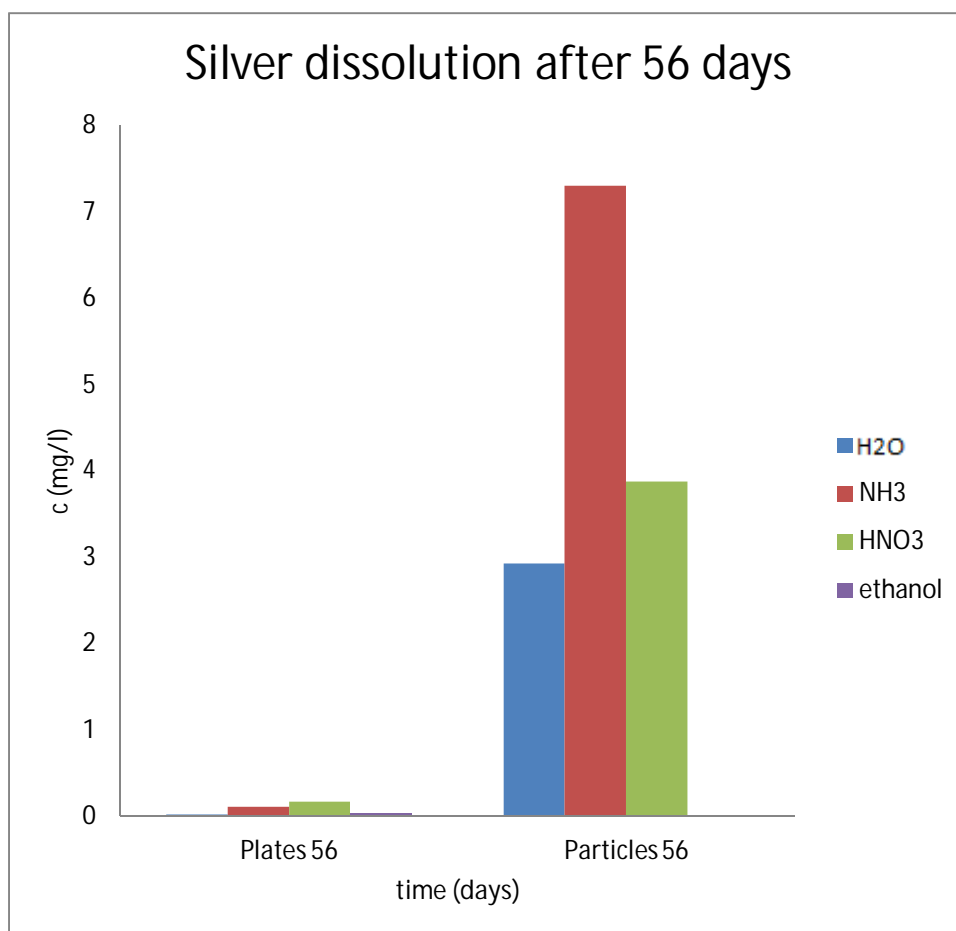
Figure 20. Silver ion concentration in water, ammonia, nitric acid solution and ethanol after 56 days of exposure.

6.5 Total silver on the surfaces

The total silver on the superhydrophobic surfaces was determined by dissolving all the silver to the solution and measuring silver ion concentraton of the solution by atomic absorption spectrometer (AAS). The pH of the nitric acid solution was the same that was used in the silver dissolution tests described previously (pH 1) and samples were exposed to the solution for 12 weeks. The scanning electron microscopy images and EDS analysis confirmed that all silver was dissolved. The procedure was repeated three times to eliminate the error of the measurement. The results are shown in Table 10.

Table 10. Silver concentration in nitric acid solution of pH 1 after 12 weeks of exposure.

Sample	Silver concentration (mg/l)
1	0,145
2	0,128
3	0,189

The results show that the amount of silver varies between different samples at some extent. This is good to notice also when comparing the dissolution of silver in the different solutions. The difference can be minimized by paying attention to the concentration of the solution during Tollens process and the speed (rpm) and duration of spin-coating.

6.6 Chemical durability of superhydrophobic silver containing coatings

Two different methods were used to investigate the chemical durability of the superhydrophobic silver containing coatings. Scanning electron microscopy was used to determine the morphology of the surface after exposure to solutions with different pH values. Water contact angle measurement was used to inspect the functionality of the surface after exposure to solutions with different pH values. The results of the chemical durability tests are shown in this chapter.

6.6.1 Analysis by Scanning Electron Microscopy

Chemical durability was determined using scanning electron microscopy. Figures 21 - 23 show that surface morphology is destroyed after 8 weeks of exposure in all of the tested solutions. The sample that was kept in de-ionized water is best preserved but it can be seen that the morphology has also changed. Morphology of the coating exposed to nitric acid was completely destroyed and only the steel plate was left as can be seen from the SEM images. The low durability of the coatings is possibly caused by the low stability of gamma-aluminum oxide.

Superhydrophobic coating with silver particles after exposure of 8 weeks to nitric acid is shown in Figure 21 a - e at different magnifications. The coating has dissolved completely and some signs of corrosion on the stainless steel can be observed at larger magnifications. The surface has inadequate acid resistance for long term immersion exposure.
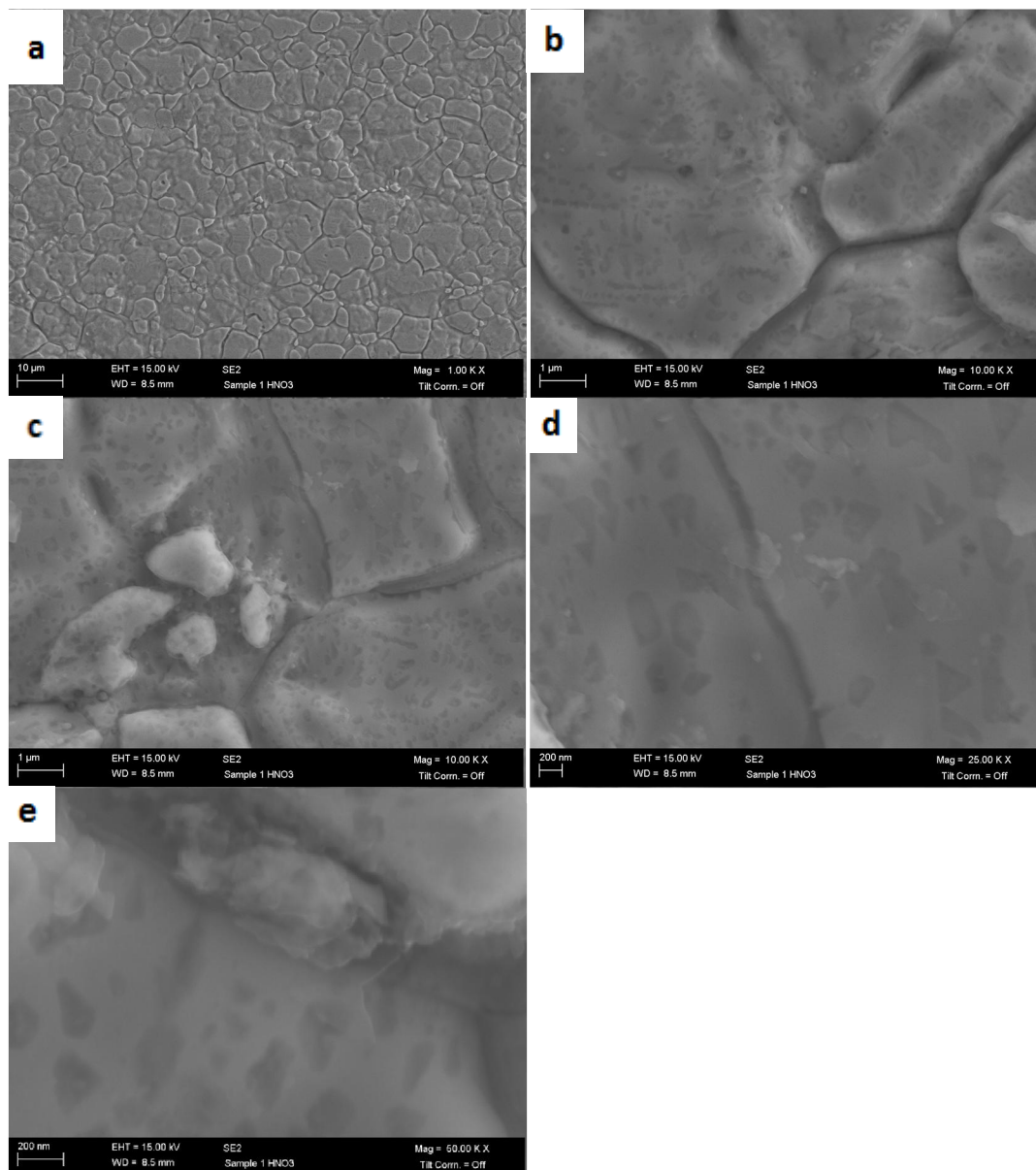

Figure 21 a - e. Silver containing superhydrophobic coating after 8 weeks of exposure to nitric acid at different magnifications.

Superhydrophobic coating with silver particles after exposure of 8 weeks to de-ionized water is shown in Figure 22 a - e. The smallest magnification shows that there are silver particles left on the surface. Larger magnifications point that the morphology of the surface has changed but the flaky morphology has partly preserved. According to the images it can be concluded that the surface has relatively good resistance against water.
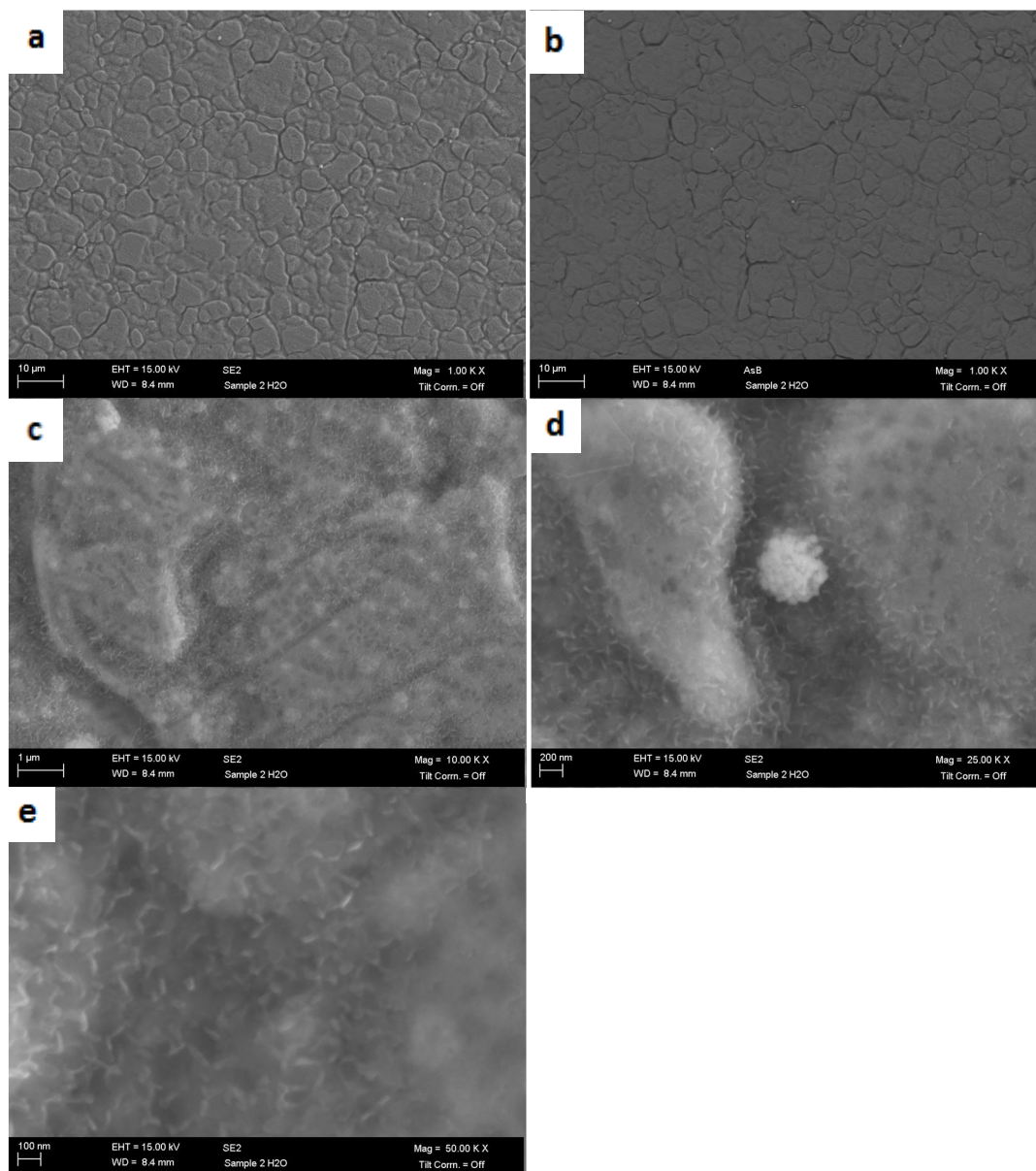

Figure 22 a - e. Silver containing superhydrophobic coating after 8 weeks of exposure to de-ionized water at different magnifications.

Superhydrophobic coating with silver particles after exposure of 8 weeks to ammonia is shown in Figure 23 a - e at different magnifications. The surface morphology has changed but the coating has not dissolved. There is no sign of silver particles in the images.
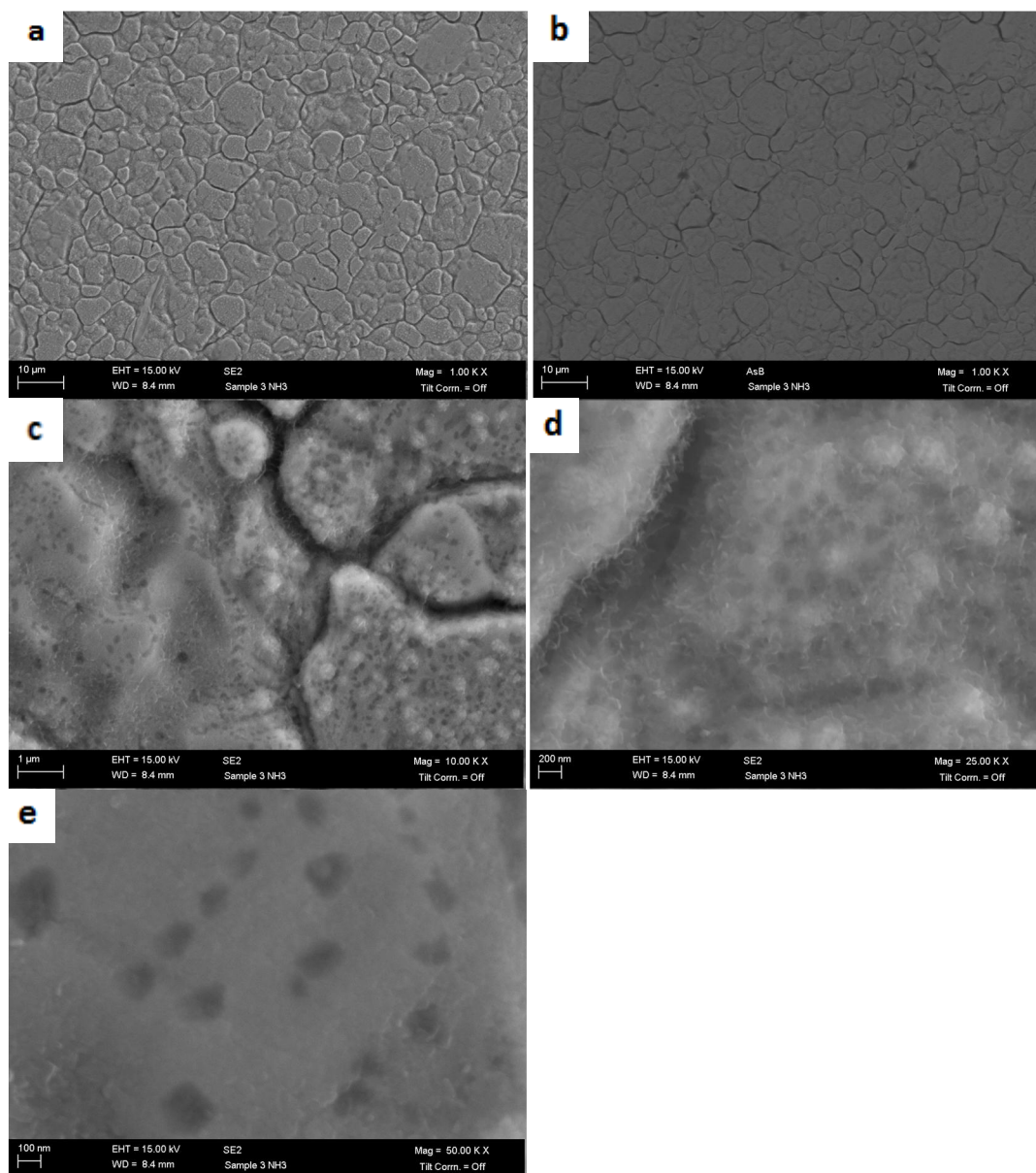

Figure 23 a - e. Silver containing superhydrophobic coating after 8 weeks of exposure to ammonia at different magnifications.

Tables 11 - 13 show EDS analysis results of the samples that were exposed to nitric acid, de-ionized water and ammonia solution. Silver was found only from the sample that was exposed to de-ionized water. Figures 24 - 26 show the areas that the EDS analyses were performed from.

Table 11. EDS analysis of silver containing superhydrophobic surface after exposure to nitric acid for 8 weeks. Results are in w-%.

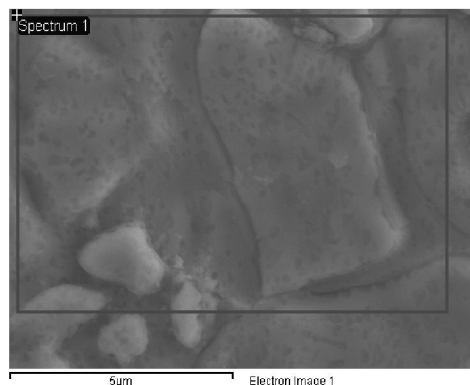| Spectrum	Ag (%)
Spectrum 1	-0,17

Figure 24. EDS analysis of silver containing superhydrophobic surface after exposure to nitric acid for 8 weeks.

Table 12. EDS analysis of silver containing superhydrophobic surface after exposure to de-ionized water for 8 weeks. Results are in w-%.

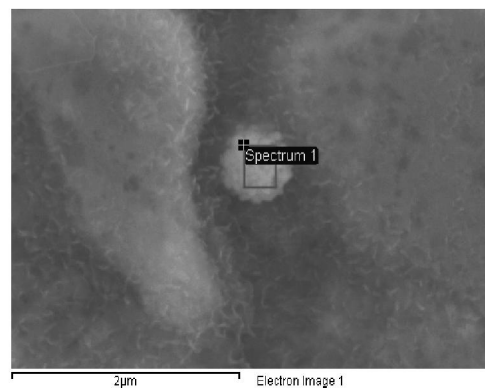| Spectrum	Ag (%)
Spectrum 1	64,7

Figure 25. EDS analysis of silver containing superhydrophobic surface after exposure to de-ionized water for 8 weeks.

Table 13. EDS analysis of silver containing superhydrophobic surface after exposure to ammonia solution for 8 weeks. Results are in w-%.

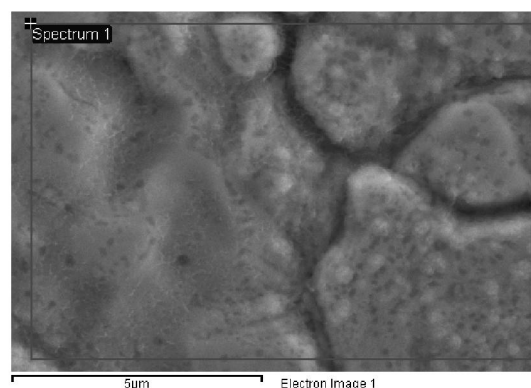| Spectrum	Ag (%)
Spectrum 1	0,0

Figure 26. EDS analysis of silver containing superhydrophobic surface after exposure to ammonia solution for 8 weeks.

6.6.2 Water contact angle measurement

The chemical durability of the superhydrophobic coatings with added silver particles in aqueous environment and with altering pH value and in ethanol was detected by measuring the water contact angle of the surfaces after exposure to de-ionized water and nitric acid, ammonia solution and ethanol. Five droplets of water were measured and the average of the results was calculated. The results are shown in Table 15. The initial water contact angle for superhydrophobic surface with added silver was 144°. The initial droplet on the surface is shown in the Figure 30. Figure 31 presents the water contact angle as a function of exposure time.

Figure 30. Water droplet on the superhydrophobic surface containing silver particles.
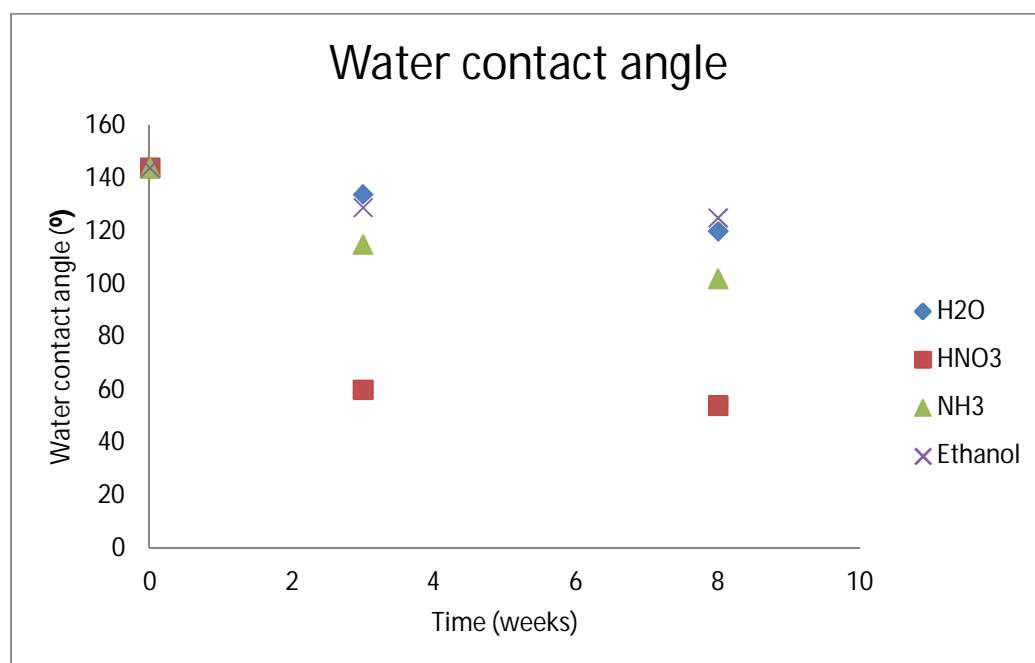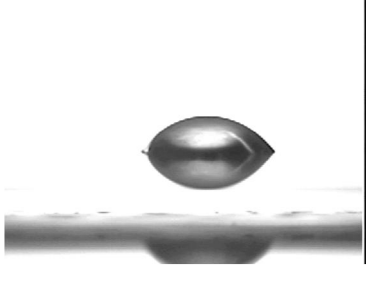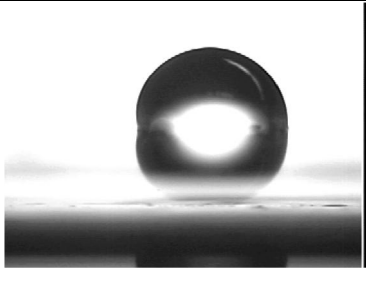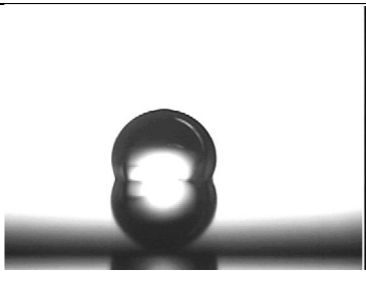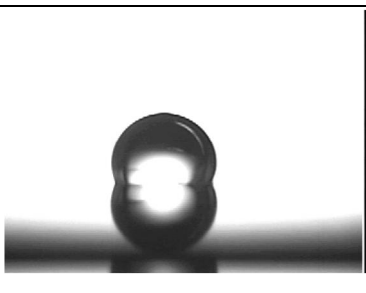

Figure 31. Water contact angle of the superhydrophobic coatings with added silver as a function of exposure time.

Table 15. Water contact angles of coatings after 3 and 8 weeks of exposure in different solutions.

Solution	Water contact angle (º) after 3 weeks of exposure	Water contact angle (º) after 8 weeks of exposure	Water droplet on the surface after 8 weeks of exposure
Nitric acid (HNO_3)	60	54	
Ammonia (NH_3)	115	102	
De-ionized water (H_2O)	134	120	
Ethanol (C_2H_5OH)	129	125	

The water contact angle measurement results show that superhydrophobic surfaces do not have durability against any aqueous environment. Durability was lowest in acidic (HNO_3) environment but coating was damaged also in alkaline (NH_3) solution and de-ionized water after eight weeks exposure. The low durability in nitric acid results from the reaction between aluminum oxide and nitric acid. When nitric acid

passes fluorosilane layer it may react with aluminum oxide forming aluminum nitrate ($Al(NO_3)_3$). Water contact angle decreases also after eight weeks exposure in ethanol.

6.7 Mechanical durability of the superhydrophobic coatings

Mechanical durability was tested with nanoscratch testing at Manchester Metropolitan University by Professor Peter Kelly's group. Figure 27 a - b shows the result of nanoscratch test.
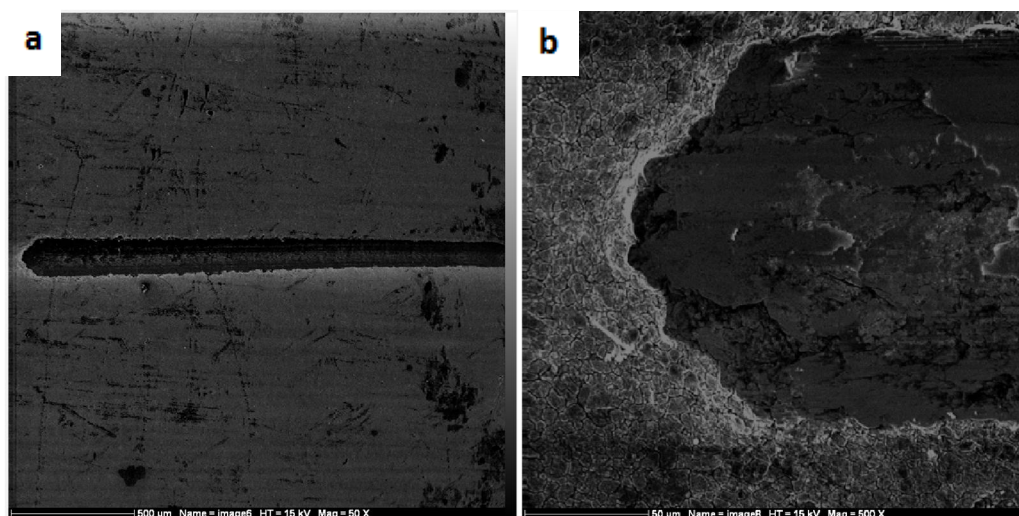
Figure 27 a - b. The scratch test result at two different magnifications. The scratch has destroyed the coating completely from the scratch area.

Because of the softness of the substrate material the blade has removed the coating. However, it cannot be seen if the coating has peeled off from the edges.

Mechanical endurance of the coatings was tested also by wiping the surface with a cloth that had been immersed to ethanol. Water contact angle of the surface was measured after wiping and it was discovered that the water contact angle had decreased from 144 ° to 113°.

A wiped superhydrophobic surface was coated again with FAS to see if the change in water contact angle was caused by removal of FAS layer during wiping. In that case the water contact angle should return to the same level as it was before wiping. However, water contact angle did not rise to the initial level after coating with new FAS layer. This indicates that some changes in the surface morphology have occurred.

SEM images (Figure 28) of the wiped surface showed that the morphology of the surface had not changed notably but there were some areas without coating that were found.
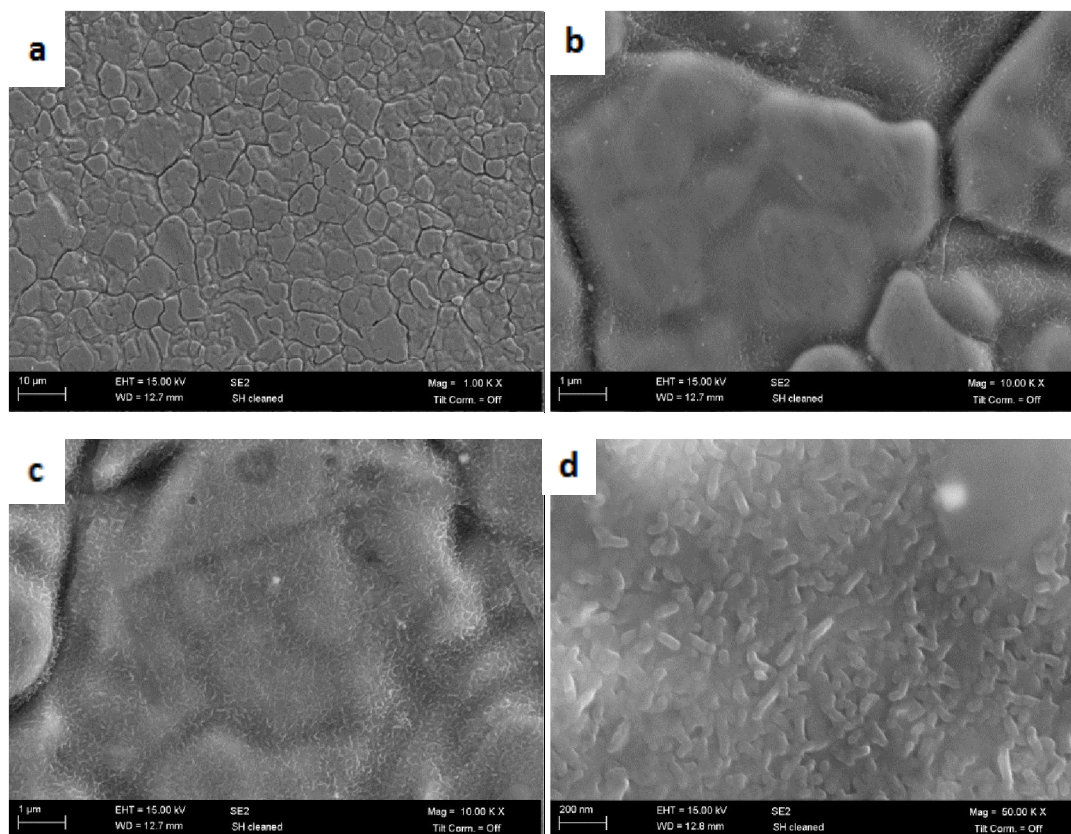
Figure 28 a - d. Scanning electron microscopy images of superhydrophobic surface after wiping with a cloth at different magnifications. Reference picture is at the page 36.

Figure 29 shows water droplet on the superhydrophobic surface before wiping, after wiping and after wiping and addition of another FAS layer on the surface. Water contact angles before wiping, after wiping and after wiping and addition of another FAS layer on the surface are shown in Table 14. Three drops of water were measured and the average of the results was calculated. Scanning electron microscopy was used to detect if surface structure had changed.
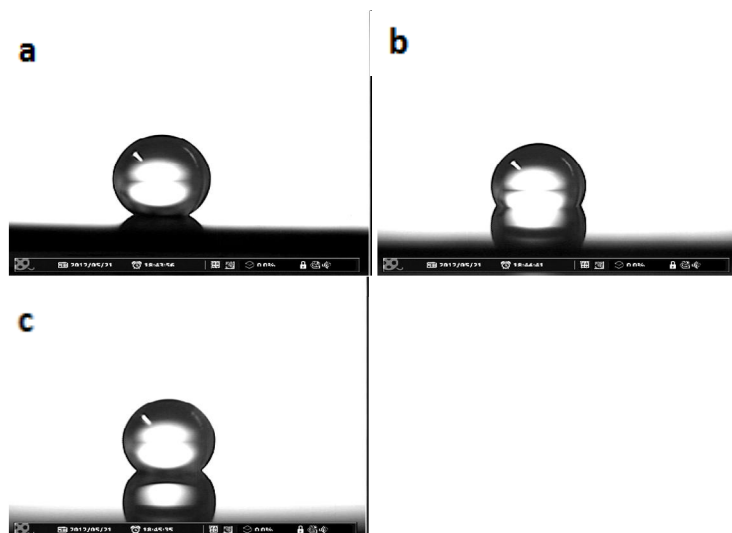
Figure 29 a: Water droplet on superhydrophobic surface b: Water droplet on wiped superhydrophobic surface c: Water droplet on a wiped surface with a new FAS layer on the surface.

Table 14. Water contact angle of superhydrophobic coating before wiping, after wiping and after coating the wiped surface with fluorosilane layer.

	Initial	Wiped	Wiped+FAS
1	136,0	108,1	129,8
2	135,2	110,4	129,0
3	141,2	119,6	128,1
Average	**137,5**	**112,7**	**129,0**

6.8 Microbiological results

Microbiological tests were carried out at VTT Technical Research Centre of Finland. Superhydrophobic surface with and without added silver (T and Ag) absorbed the dye used for the epifluorescence microscopy in such a high quantity that distinguishing the bacteria from background was impossible.

The number of attached bacteria on superhydrophobic coating with and without added silver was determined by cultivation. The results show that superhydrophobicity did not prevent bacteria from adhering. However, it is not known if the adhesion force of the bacteria is lower compared to the adhesion force of the bacteria on a surface that has lower water contact angle. Superhydrophobic surface without silver did not kill the adhered bacteria. However, the coating with added silver killed the majority (88%) of adhered bacteria. Figure 54 shows the bacterial cell count (cm^{-2}) of biofilm formed on superhydrophobic coating with and without silver and AISI 304 stainless steel reference plate. [68]
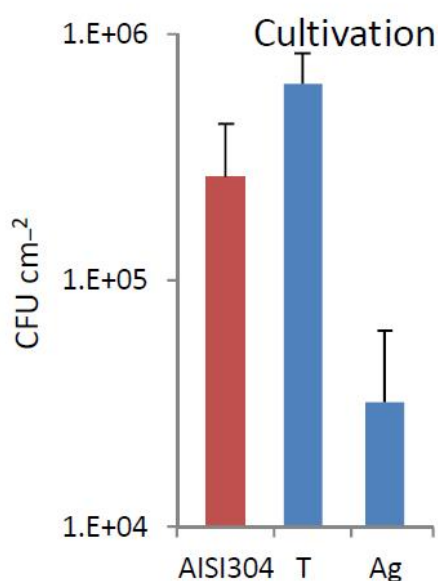

Figure 32. Bacterial cell count (cm^{-2}) of biofilm formed on superhydrophobic coating with and without silver and AISI 304 stainless steel reference plate. [68]

6.9 Hybrid surfaces with superhydrophobic and photocatalytis properties

Superhydrophobicity of the TiO_2 hybrid surface was tested measuring water contact angle of the surface. A high water contact angle of 136⁰ was measured (Figure 33). The rolling angle of the surface was less than 10 ⁰.
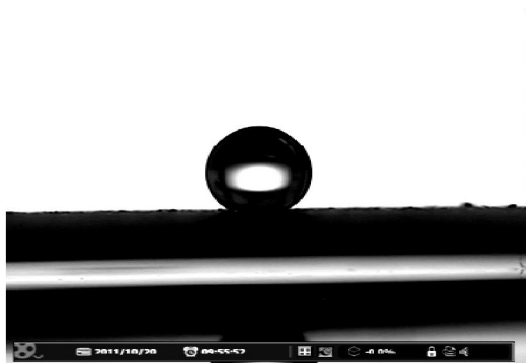
Figure 33. Water droplet on a hybrid surface.

Photocatalytic activity of the surface was measured using Resazurin ink test. The ink was spread on the surface and it was then irradiated two minutes with UV light. The test showed that the titanium dioxide layer was photoactive as the ink reduced changing color on the surface during irradiation (Figure 34 a). However, when the fluorosilane layer was added the color of the ink remained blue and thus the surface was no longer photoactive (Figure 34 b).
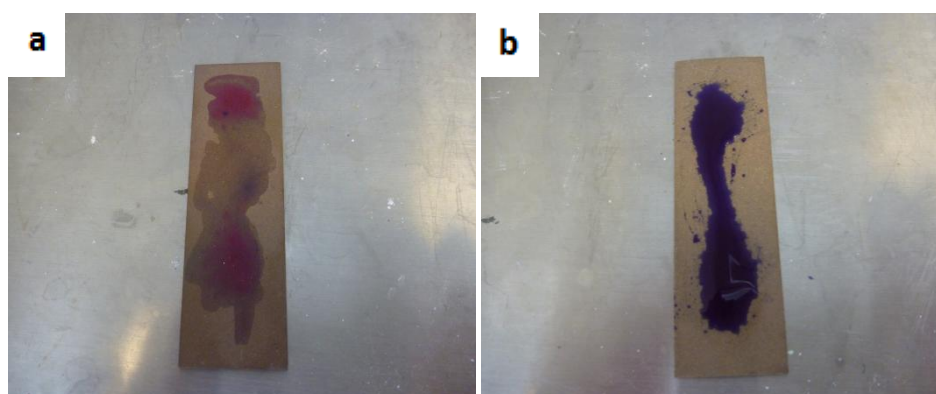
Figure 34 a: Resazurin test for a titanium dioxide coating. The color of the ink changed from blue to red after two minutes of irradiation with UV light. b: On a surface with a top fluorosilane layer the color of the ink did not change after two minutes of UV irradiation.

Mechanical durability of the coating was tested rubbing the surface with a cloth that was immersed in ethanol. After wiping the coating was dried in air and water contact angle was measured. The water contact angle decreased significantly (Figure 35) as the water contact angle was measured to be 71°.

Figure 35. Water droplet on a hybrid surface that has been wiped with ethanol.

To find out if the change in water contact angle was due to a reaction between surface and ethanol the hybrid coating was immersed in ethanol without wiping. However the water contact angle remained the same as the initial water contact angle (136 ^{o}). These results indicate that the reason of the low mechanical endurance of hybrid coatings is caused by low adhesion of the fluorosilane layer to the substrate material.

7 Conclusions

7.1 Dissolution of silver

The results show that silver dissolves from commercial particles as well as from superhydrophobic coatings at some extents in all aqueous solutions regardless of the pH of the solution. Dissolution rate is highest with high and low pH values but solubility occurs also in pure de-ionized water. It can be seen from the results that the amount of dissolved silver is high enough to cause antibacteriality in all aqueous environments.

The solubility rate of silver from silver containing superhydrophobic surfaces was highest in nitric acid solution whereas the dissolution rate of silver from commercial silver nanoparticles was highest in ammonia solution. The amount of dissolved silver from superhydrophobic surfaces cannot be compared straightly to the results of solubility of silver nanoparticles as the initial amount of silver may have varied slightly between the coatings due to the silver plating method. Other factor that may have affected to the higher solubility of silver in nitric acid compared to ammonia is the possibility that nitric acid has destroyed the top fluorosilane layer of the coating faster and thus exposed silver particles to the solution. The solubility of silver from the coatings in de-ionized water was low but almost linear which indicates that silver dissolves evenly from the surface. After eight week of exposure to the de-ionized water some silver was detected from the surface. In nitric acid and ammonia all silver was gone after eight weeks and thus they are not suitable for applications requiring conservation of the antibacterial property.

7.2 Antibacterial activity of silver

It was shown that superhydrophobic coatings which contain small silver particles prevent the growth of micro-organisms. On the contrary, superhydrophobic coatings without silver did not show tendency to kill micro-organisms. However, superhydrophobicity may be advantage when destroyed micro-organims are removed from the surface.

Microbiological test results indicate that antibacterial activity is caused by dissolved silver. However, the rate of silver dissolution should not be too high so that the antibacterial effect would last longer.

7.3 Durability of the coatings

The results showed that the superhydrophobic alumina based coatings cannot preserve their properties in acidic and alkaline solution. Solution is able to penetrate through the top FAS layer and gamma aluminum oxide is dissolved and thus the structure of the coating is destroyed. The dissolution behavior of the coating in deionized water is similar but notably slower. FAS layer does not cover silver particles and when the silver particles dissolve water reaches alumina layer through the holes from which silver dissolved.

Mechanical durability of the superhydrophobic alumina based coatings was also relatively poor since the coating could not endure the scratching tests or wiping with a cloth. That seems to arise from the low adhesion of the low energy organic layer on the top of the surface and partly from the destruction of the surface morphology after exposure to mechanical load. The nanoscratch tests showed also that the coating is destroyed under mechanical loading as the the AISI 304 steel substrate is relatively soft and thus substrate cannot endure the stress.

TiO_2 surfaces lost also their hydrophobic property after wiping with a cloth. This supports the suspicion that the decrease in the water contact angle after wiping is caused by either the removal of fluorosilane layer or the change in topography of the surface.

7.4 Future work

According to the results achieved in this work the superhydrophobic coatings with silver particles could be used in applications that require antibacteriality. However both chemical and mechanical durability of the coating set limits for the applications. The low chemical durability in aqueous solution is probably caused by the solubility of gamma aluminum oxide. If water gets through the top FAS layer the alumina layer dissolves and flaky structure that causes superhydrophobicity disappears.

In addition to the durability problems, manufacturing process of superhydrophobic surfaces is also relatively complicated and the size of the coated object is limited. A solution to these problems could be changing the materials and trying to maintain the structure that lowers surface-energy. Titanium dioxide is more chemically stable material in aqueous solutions compared to gamma aluminum oxide. However, the titanium dioxide coatings tested in this thesis did not show better mechanical endurance compared to aluminum oxide based superhydrophobic surfaces. The structure of the material, in this case titanium dioxide or some other stable material,

should be changed so that the poorly attached organic top layer that lowers surface energy would be replaced by a structure that has low enough surface energy without any organic layer on the top. There are some studies showing high water contact angle results for metal oxides that have nanorod structures. That is one possibility that should be considered when finding new solutions.

Use of titanium dioxide or other photocatalytic material makes it possible to increase antibacteriality. However, the addition of photoactivity to the surface gives rise to many challenges. If low surface energy that is required to obtain superhydrophobic surface is obtained with organic film, that layer may be degraded in a photocatalytic reaction on the surface.

Nonetheless, for applications that do not get heavy mechanical load or hard environmental conditions, the initial coating studied in this work has proved to be an efficient and competitive choice.

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