The Mechanism of Silver Dissolution for Biomedical Devices and Hygienic Coating Applications.

Yanmei Ma
Dublin Institute of Technology

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The Mechanism of Silver Dissolution for Biomedical Devices and Hygienic Coating Applications

Yanmei Ma (MSc.)

The thesis is submitted to Dublin Institute of Technology for the award of Ph.D.

Supervisors:
Dr. Tony Betts and Dr. John Cassidy

August 2008

School of Chemical and Pharmaceutical Sciences
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I wish to acknowledge the financial support of the HEA TSR Strand 1 Scholarship.
Declaration

I certify that this thesis, which I now submit for examination for the award of PhD, is entirely my own work and has not been taken from the work of others unless cited and acknowledged within the text of my own work.

This thesis was prepared according to the regulations for postgraduate study by research of Dublin Institute of Technology and has not been submitted in whole or in part for an award in any other Institute or University.

The work reported on in this thesis conforms to the principles and requirements of the Institute’s guidelines for ethics in research.

Signature__________________
Date____________________
Yanmei Ma
Abstract

This work focuses on the mechanism of silver dissolution for biomedical and hygienic coating applications. The research work began from the investigation of silver dissolution behaviour from pure silver and silver-platinum alloys in solutions with or without chloride using the technique of cyclic voltammetry (CV). A Zero resistance amperometry (ZRA) measurement was carried out to study the effect of platinum on the silver dissolution behaviour in 0.89%wt. NaCl solution. The mechanism of silver dissolution from pure silver and silver-platinum alloys in 0.89%wt. NaCl solution was proposed by an equivalent circuit model under the investigation of electrochemical impedance spectroscopy (EIS). It was found that the addition of Platinum decreased the kinetics for silver oxidation observed by CV and EIS.

Thin silver and silver-platinum alloy coatings on polymeric materials were formed using a sputter coater with an argon plasma source. Silver dissolution from the plasma coatings on the polymeric material showed a similar behaviour to bulk silver or silver-platinum alloys investigated by CV in 0.89%wt. NaCl solution.

Novel polymeric composites containing silver nanoparticles were prepared by the reduction of AgNO₃ with NaBH₄ under the protection of polymers. The properties of the silver nanoparticle polymeric composites were characterized by UV-vis spectroscopy, X-ray diffraction technique, as well as Raman spectroscopy. Silver dissolution behaviour from the polymeric silver nanoparticle composite was investigated by CV in 0.89%wt. NaCl. The antibacterial activities against strains of E. coli (Gram-negative) and S. aureus (Gram-positive) of silver nano-particles polymeric composite were investigated. The Alamar Blue(AB) assay and the Neutral Red (NR) assay were employed to evaluate the cytotoxicity of the polymeric silver nanoparticle composite to A549 cells. The silver nanoparticles stabilized with PVA crosslinking with adpic acid showed high antibacterial activity without causing serious cytotoxicity.
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Chapter 1 Introduction
Chapter 1 Introduction

Abstract

This chapter describes the properties of the biomaterials, such as polymers, metals and ceramics and their applications in medical devices and hygienic coatings. The use of silver in medicine and/or medical devices is concisely reviewed. Finally, the aims of the project elaborating the mechanism of silver dissolution for biomedical and hygienic coatings applications are described.
1.1 Biomaterial

1.1.1 Definition of a biomaterial

A biomaterial is a synthetic material used to replace part of a living system or to function in intimate contact with living tissue [1]. Biomaterials are used in implants or to convey and process body fluids such as blood [2]. Standard design considerations may apply, such as strength and deformation, fatigue and creep, friction and wear resistance, flow resistance and pressure drop, thermal stability and expansion, electrical conductivity, optical transparency and refractive index. In addition, the material must be biocompatible and able to withstand cleaning and sterilization to minimize the risk of infection. A material is biocompatible if it evokes a minimal adverse biological response. This includes the effect of the biological environment on the material, as well as the effect of the material on tissue.

1.1.2 Classification of biomaterials and their properties

Materials for biological use are classified according to their base structure such as ceramics, composites, metals and polymers. Table 1.1 summarizes different types of biomaterials and their most common applications. Table 1.2 illustrates the most common polymers and their clinical uses.
Table 1.1 Classification of biomaterials in terms of their base structure and some of their most common applications (Types of biomaterials)[1]

<table>
<thead>
<tr>
<th>Biomaterials</th>
<th>Applications</th>
</tr>
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<tbody>
<tr>
<td>Ceramics</td>
<td>Dental and orthopedic</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Aluminium oxide</td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td></td>
</tr>
<tr>
<td>Composites</td>
<td>Heart valves and joint implants</td>
</tr>
<tr>
<td>Carbon-carbon fibers and matrices</td>
<td></td>
</tr>
<tr>
<td>Metals</td>
<td>Joint replacement components,</td>
</tr>
<tr>
<td>Pure metals</td>
<td>Fracture fixation, dental implants,</td>
</tr>
<tr>
<td></td>
<td>Pacemakers, suture wires,</td>
</tr>
<tr>
<td></td>
<td>Implantable electrodes</td>
</tr>
<tr>
<td>Aluminium</td>
<td></td>
</tr>
<tr>
<td>Chromium</td>
<td></td>
</tr>
<tr>
<td>Cobalt</td>
<td></td>
</tr>
<tr>
<td>Gold</td>
<td></td>
</tr>
<tr>
<td>Iridium</td>
<td></td>
</tr>
<tr>
<td>Iron</td>
<td></td>
</tr>
<tr>
<td>Manganese</td>
<td></td>
</tr>
<tr>
<td>Molybdenum</td>
<td></td>
</tr>
<tr>
<td>Nickel</td>
<td></td>
</tr>
<tr>
<td>Niobium</td>
<td></td>
</tr>
<tr>
<td>Palladium</td>
<td></td>
</tr>
<tr>
<td>Platinum</td>
<td></td>
</tr>
</tbody>
</table>
Tantalum
Titanium
Tungsten
Vanadium
Zirconium

Metallic alloys
Wide variety using the above metals

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly (ethylene) (PE)</td>
<td></td>
</tr>
<tr>
<td>Low density (LDPE)</td>
<td>Bags, tubing</td>
</tr>
<tr>
<td>High density (HDPE)</td>
<td>Non-woven fabric, catheter</td>
</tr>
<tr>
<td>Ultra high molecular weight (UHMWPE)</td>
<td>Orthopedic and facial implants</td>
</tr>
<tr>
<td>Poly methyl methacrylate (PMMA)</td>
<td>Intraocular lens, dentures, bone cement</td>
</tr>
</tbody>
</table>

Table1.2 The clinical uses of some of the most common biomedical polymers related to their chemical structure and physical properties[1]
Chapter 1 Introduction

<table>
<thead>
<tr>
<th>Material</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly vinyl chloride (PVC)</td>
<td>Blood bags, catheters, cannulae</td>
</tr>
<tr>
<td>Polyethylene terephthalate (PET)</td>
<td>Artificial vascular graft, sutures, heart valves</td>
</tr>
<tr>
<td>Poly esters</td>
<td>Bioresorbable sutures, surgical products, controlled drug release</td>
</tr>
<tr>
<td>Poly amides (Nylons)</td>
<td>Catheters, sutures</td>
</tr>
<tr>
<td>Polyurethanes (PU)</td>
<td>Implant coatings, film, tubing</td>
</tr>
</tbody>
</table>

Materials for implanted devices should be resistant to corrosion or attack by body fluids. Although galvanic and stress corrosion are small, metals are not completely inert. Yet metallic pins and plates, for example, have been used for many years. Ceramics are resistant to corrosion in the tissue environment. They have good strength in compression and are hard, and thus wear resistant, but brittle. In most respects, ceramics are inert in the biological environment.

Polymers tend to weaken as a result of chemical action with time, and swelling and leaching may be observed through migration of fluid into the polymers or migration of solid or liquid components out into the tissue. High-density polyethylene is inert and resistant to deterioration, as is polypropylene. Polyesters such as polyethylene are susceptible to hydrolysis and loss of tensile strength, but Dacron cloth is used for suturing heart valves into tissue. Polyamides (nylon) absorb water and irritate tissue and may lose tensile strength. Silicone rubber (e.g., Silastic, dimethyldichlorosilane rubber with silica filler and polymerized by stannous octate), which is inert and little affected by tissue and body fluids, is
used in many medical devices. Polytetrafluoroethylene (PTFE) (Teflon) is inert and not affected in solid form, but small particles may cause tissue irritation. Polymethylmethacrylate (PMMA Plexiglass or Lucite or PMMA adhesive), which is an acrylic, may be deteriorated by heat sterilization (exhibiting crazing and/or loss of strength). The adhesive or cement is often used to fasten metallic joint replacements into bone, but there is appreciable heat production during curing. Sutures are of two types: absorbable and non-absorbable. The absorbable types are catgut or chromic treated catgut (basically collagen); they have little tensile strength after a few days. The non-absorbable type includes silk, polyamide, polyethylene terephthalate, and PTFE. Strength is well maintained, except by nylon multifilament, which has little strength after 6 months (and also causes a significant tissue reaction). Composite (e.g., cermets, ceramic plus metal) should be similar in chemical resistance to each of the components. However, a ceramic-coated metal (may be desirable for wear resistance or low friction) should respond like a ceramic. Composites of fibers, strong in tension, with a matrix strong in compression, combine strength, toughness, and stiffness and may permit parts to be made lighter or stronger.

1.1.3 The effects of biomaterials on tissue

It is necessary to evaluate potential response of tissue in contact with biomaterials. These include thrombosis and hemolysis, inflammation and adaptation, infection, carcinogenesis, and hypersensitivity. This section mainly introduces the issue of infection.
Chapter 1 Introduction

It has been stated that there is a race between regenerating tissue and bacteria for sites on or near an implant. Infection of implanted biomaterial is serious and usually requires surgical removal of the implant. In addition to being clean and pyrogen free, an implant (or any material contacting a wound or blood) must be sterile. Methods of sterilization are as follows: cold solution, dry heat, moist heat (steam), gas, and radiation. Cold (room temperature) solution uses commercial preparations, such as cidex, which usually contain formaldehyde or glutaraldehyde. The part is allowed to soak 1 to 3 hours. Dry heat consists of heating at 160 °C - 175 °C for 0.5 to 2 hours (time and temperature vary inversely). Moist heat is applied in an autoclave; temperature is at 120 °C - 130 °C for 2 to 15 minutes, varying inversely (higher temperature for a shorter time). Gas sterilization is usually performed with ethylene oxide, 400 to 1200mg/L, 1 to 24 hours, from room temperature to 55 °C. Radiation may be via energetic electron or gamma rays, e.g. 2 to 4 Mrad, at room temperature. Irradiation is performed by the manufacturer and the material is sealed in a container until ready for use. The choice of sterilizing method depends on its effect on the biomaterial, as well as time required or convenience. However, all these method may not be suitable for a material implanted into human bodies and sterilizing in-situ. Thus a novel biomaterial that is resistant to infection is required in medical applications, for example, silver contained biomaterial used in the urinary catheters.

1.2 Silver used in biomedical applications
1.2.1 History of silver in biomedical applications

Silver is a powerful, natural prophylactic/antibiotic, used for thousands of years. Ancient Greeks lined their eating and drinking vessels with silver, as did many other cultures throughout the world [3]. Pioneers of the American West would put a silver dollar in a jug of milk to keep it fresh without refrigeration [4]. Silver, e.g. in the form of hardened silver nitrate (lunar caustic), has long played a role in the treatment of burns. In the 18th century, lunar caustic as lapis infernalis was commonly called then, was already used to remove granulation tissue from wounds which were healing slowly or not at all. In the 19th century, lapis infernalis had won a permanent place for itself for this use. Argentis nitras is the best of the mildest caustics. Its utility for stimulating indolent ulcers, and keeping granulations from rising too high, is well-known to everyone. Silver nitrate was also applied to fresh burns early in the 19th century. It was used in all kinds of forms, e.g. solid (as a rod or pencil used to dab the wounds) or as a solution in concentrations varying from 0.2% to 2%. As bacteriology developed towards the end of the 19th century, the antibacterial properties of silver (e.g. in metallic form) were subjected to investigation. Silver nitrate solution was used to prevent eye infections caused by gonococci in newborn infants [5].

Scientific experiments conclude that silver works on the full spectrum of pathogens without any side effects or damage to the body. Silver does more than kill disease-causing organisms. It also causes major growth stimulation of injured tissues. Burn patients and even elderly patients notice more rapid healing. All strains of pathogens resistant to other antibiotics are killed by silver [6].
Other attempts were also made to utilize the antibacterial effect of silver by producing a continuous flux of silver ions which was not time-dependent, as that produced by silver sulphasadiazine. It might be expected that the antibacterial effect would then be greater. Two silver nylon products were investigated in this context: silver nylon (SN) containing 26% metallic silver and woven heavy rip stop (HRS) containing 20% metallic silver. In vitro studies showed that these silver-coated nylons were active against many microorganisms [7]. Studies were carried out and aimed at the possibility of increasing the concentration of silver ions at the wound surface by means of electrolysis [8]. The combined effect of electric current and the silver-coated nylons was investigated in an in vitro study. The anti-bacterial effect of silver nylon and HRS was found to be markedly increased by application of a weak direct anode current, due to an increase in the release of silver by these materials.

1.2.2 Silver containing biomaterials

1.2.2.1 Silver-carbon biomaterials

One of the applications of activated carbon fiber is its use as a water purifier and the carbon material is known to have a high affinity for bacteria. However, bacteria may breed on this fiber during the purification process, becoming itself a pollutant. In order to avoid such a disadvantage, activated carbon fiber containing silver materials were prepared and studied for antibacterial purpose [9-15].
An activated carbon fiber supporting silver (ACF/Ag) was tested for its antibacterial capacity against *Escherichia coli* (*E. coli*). Water that has passed through ACF/Ag demonstrated strong bactericidal ability. This activity decreased over the time suggesting that generated bactericidal species had a short lifespan [14], [16]. Park et al. [17] investigated the effect of immersion in silver nitrate solution on activated carbon fibers (ACFs) in relation to adsorption behavior and antibacterial activity of ACFs supported with silver (ACF/Ag). The silver content of ACF/Ag increased with increasing concentration of silver nitrate. It was found that the micro-pore structure slightly decreased as the silver nitrate concentration increased. Otherwise, it was revealed that the ACF/Ag possessed a strong antibacterial activity and an inhibitory effect for the growing of *E. coli* and *S. aureus*, respectively. Silver content on ACF/Ag decreased rapidly because of rough morphology of silver particles in water erosion.

Pitch-based activated carbon fiber (ACF) supporting silver (ACF/Ag) was prepared by pre-oxidization, carbonization, activation, immersion and decomposition processes. The ACF exhibited increased oxygen-containing functional groups and decreased BET specific surface areas after supporting silver particles. The ACF/Ag with C-OH, C=O, -COOH and -COOAg groups showed strong adsorption and antibacterial activity against *E. coli*, which suggests it is a promising antibacterial material. However, ways of controlling the distribution of silver-particle size should be found before any practical application.[9]

Silver acetate dissolved in quinoline was mixed thoroughly with the spinnable isotropic petroleum pitch dissolved in the same solvent. After removing the quinoline, the resulting pitch was spun, stabilized, carbonized, and finally activated at 900 °C under a stream of steam. The fiber with 33 wt% yield after
activation contained 0.63 wt% of fine silver particles and showed N₂-BET specific surface area of 740 m²/g. It showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, before and after soaking in flowing tap water for 20 days. How to improve spinnability of the pitch containing silver acetate and how to increase the specific surface area effectively by the activation process are problems remaining to be solved [10].

Methyl methacrylate-grafted phenolic resin fiber was soaked in AgNO₃ aqueous solution to support Ag⁺ by ion exchange reaction. The fiber supporting Ag⁺ was carbonized at 900 °C for 30 min in a nitrogen atmosphere, followed by activation at 900 °C under a stream of steam. One of the activated fibers contained 0.58 wt% of silver and showed 2100 m²/g of specific surface area and 200 MPa of tensile strength. This fiber showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* before and after soaking in the flowing tap water for 20 days [11].

Phenolic resin containing cobalt as an activation catalyst and silver as an antibacterial agent was spun, stabilized, carbonized and activated in steam. The number of the metal particles increased as activation proceeded and reached to 100-200 nm in diameter at the largest. The activated carbon fiber with a mesopore surface area of 72 m² g⁻¹ was obtained after 51 wt% burn-off of the carbon fiber containing 0.22 wt% Ag and 52 ppm Co. The silver somewhat disturbed the formation of both micro- and mesopores through catalytic activation by cobalt. A silver content of 0.22 wt% in the activated carbon fiber was rapidly decreased to 0.0006 wt% after immersion in flowing tap water for 20 days, but the resulting fiber exhibited antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [12]. A type phenolic resin including 1 wt% of AgNO₃ was spun,
hardened, carbonized at 900 °C under nitrogen and finally activated at 900 °C for 10 min and 60 min under steam. The resulting fibers showed specific N₂-BET surface areas of 280 m²/g and 1940 m²/g, respectively. The latter contained 2.2 wt% of the silver particles of which the largest size was several 100 nm, and showed antibacterial activity against Staphylococcus aureus and Escherichia coli. After soaking in the flowing tap water for 20 days, this fiber lost one third of silver but kept the antibacterial activity [13].

Silver-dispersed carbon aerogels (CAs) were obtained by direct immersion of organic aerogels prepared by ambient pressure drying technique in AgNO₃ aqueous solution and then carbonization. The dispersion and structure of silver nanoparticles in these materials were investigated by means of scanning electron microscopy, transmission electron microscopy and X-ray diffraction. The Ag-dispersed CAs exhibit strong and long-term antibacterial activity [15].

1.2.2.2 Ag-SiO₂ biomaterials

It has been shown that bioactive glasses provide an ideal environment for colonization, proliferation and differentiation of human osteoblasts to form new bone and silver contained bioactive glasses have been interesting materials for wound repair applications [18-21]. For example, the role of silver ions was evaluated in composite resin dental materials; the Ag⁺ filler showed significantly more antibacterial activity than the control filler without silver ions and it may be useful to add this Ag⁺ filler to composite resin dental materials for secondary caries protection [22]. Another example consists of silver-doped silica thin films successfully prepared by a sol-gel method used for antibacterial materials. The
starting solution was prepared from Si(OC$_2$H$_5$)$_4$ : AgNO$_3$ : H$_2$O : C$_2$H$_5$O(CH$_2$)$_2$OH = 1:0.24:3.75:2.2 (molar ratios) and then the pH value controlled at 3 with 0.5M HNO$_3$ solution. The formation of silver-doped glassy silica thin films at various temperatures was investigated and it was found that silver ions were completely trapped in the silica matrix and their reduction could be achieved at 600 °C annealing temperature. The antibacterial effects of silica thin films against *Escherichia coli* and *Staphylococcus aureus* were examined by a film attachment method and the coated films had an excellent antibacterial performance [23].

1.2.2.3 Ag alloy biomaterials

Very few studies have been carried out on the anti-bacterial properties of silver containing alloys. Silver and silver-platinum alloy coatings used in the biomedical devices and hygienic coatings were studied by Dowling and Betts et al [24-26]. Another silver alloy sample was aluminum-silver. Aluminum specimens were first anodized in a sulfuric acid bath, and then silver was electrodeposited into pores of the anodized aluminum by using alternating current. The anodized aluminum with deposited silver was tested for its antibacterial performance. The results show that the antibacterial rates of the specimens with deposited silver are above 95% against the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Staphylococcus aureus*. The morphology of the silver in pores of anodized aluminum was characterized by transmission electron microscopy, and the micrographs showed that silver was assembled in the form of nanowires with a diameter of 10 nm or 25 nm. The nanowires have a morphology of parallel bright stripes alternating with parallel dark stripes [27].
1.2.2.4 Ag-zeolite biomaterials

The silver containing zeolites were also studied for the biomedical applications. Some samples prepared by loading silver into zeolite by ion exchange reaction were estimated for their antibacterial activity by testing viable counts of *Escherichia coli*. This result indicated that the antibacterial activity depended on the presence of light and the dissolved oxygen [28]. The study for evaluating the antibacterial effect of silver-zeolite (SZ) against oral bacteria under anaerobic conditions was investigated and the results suggested that SZ may be a useful vehicle to provide antibacterial activity to dental materials used even under anaerobic conditions such as deep in the periodontal pocket [29]. The antimicrobial effect of the Mexican zeolitic mineral from Taxco, Guerrero, exchanged with silver ions was investigated. It was found that the Mexican silver clinoptilolite-heulandite mineral eliminated the pathogenic microorganisms *E. coli* and *S. faecalis* from water with the highest amount of silver supported on the mineral after 2 h of contact time. Under these conditions, the silver level in water remained in 50 µg L⁻¹ [30].

1.2.2.5 Ag-polymer biomaterials

Bacterial growth on medical implants and devices is a common source of infection. There is a great deal of interest in the surface modification of polymeric materials to decrease infection rates without altering properties that affects their function. One possibility is to coat the material with an antibacterial agent such as
silver [31-34]. Some work explores the feasibility of depositing adherent silver films onto biomedical poly (ether urethanes) by an electroless plating process. The surface chemistry of the deposition process and the effect of a plasma treatment on the metal/polymer adhesion were explored. The silver films produced on an unmodified poly (ether urethane) surface consist predominantly of micron-sized clusters that form in solution and are poorly adhered to the surface. However, some small adherent clusters are also deposited on the polymer surface and X-ray photoelectron spectroscopy of the metal/polymer interface shows evidence of chemical interaction between silver and surface carbonyl groups. An air plasma treatment of the polymer to increase the number of carbonyl containing groups at the surface has been shown to significantly improve the metal/polymer adhesion and to decrease the porosity of the silver films.

1.2.2.6 Nano-silver biomaterials

The study of silver nano-particles became popular, for example, the Toshima research group has prepared and studied silver nano-particles used as catalysts for the oxidation of ethylene [35-40], and as well as the research group of Jong Hak Kim [41-43]. Recently silver nano-particles combined with polymers have been studied for their applications as antibacterial materials [33,44-53].

1.3 The application of silver in biomedical devices and hygienic coatings

Silver coating of medical devices is believed to prevent device-associated infection. Several in-vitro and in-vivo studies, as well as clinical observations on silver--nylon, silver-intramedullary pins, silver oxide Foley catheters and silver-
coated vascular prostheses have been performed during the past 30 years. Nevertheless, randomized clinical studies showing efficacy of such coated medical devices in high-risk patient populations are rare, have dealt with very small numbers of patients, or remain controversial [54].

1.3.1 Application in urinary catheters

Urinary tract infections (UTIs) account for up to 40% of all hospital-acquired infections [55]. The prevention of catheter-associated urinary tract infections (CA-UTIs) with the use of silver-coated catheters (SCCs) has been studied and investigated [56,57]. Silver-coated catheters can reduce the incidence of CA-UTI in a community medical center by 65%. These catheters also offer a significant cost savings to the facility. Infection associated with an indwelling catheter is a representative type of biofilm infection occurring in the urinary tract. Since the most effective way to control this intractable infection is the prevention of bacterial attachment and subsequent biofilm formation on the catheter, the importance of catheter materials and anti-bacterial coating cannot be underestimated. The difference in the degree of bacterial attachment among standard catheter materials, the efficacy of silver-coating of catheters in preventing infection and the potency and effectiveness of a new lecithin/silver coating are discussed [58].

However, Leone [59] reviewed literature concerning the diagnostic, prevention, and management of nosocomial urinary tract infection (NUTI) in intensive care unit (ICU) patients harboring a urinary catheter. The level of evidence given by this data is weak and shows the need to make randomized studies in the field of
catheter-associated UTIS in ICU patients and questioned. Additional studies with a high standard of internal quality and careful specification of the clinical question with regard to subject-specific critical points are necessary to decide whether the use of silver-coated catheters can be recommended.

1.3.2 Application in Central venous catheters (CVCs)

Catheter-associated bloodstream infections remain an important cause of nosocomial infection, with an estimated 50,000-100,000 cases occurring each year in the United States. Central venous catheters are believed to be responsible for 90% of such infections. The cumulative risk of acquiring a catheter-related bloodstream infection has ranged between 1% and 10% for central venous catheters in general and 6% for total parenteral nutrition catheters. The skin is the most common source of organisms causing catheter-related infections. Recent prospective studies have shown that the incidence density per catheter day does not increase with duration of catheterization and that routine changes, either over a guidewire or by new site puncture, do not appear to lower the risk of infection. Diagnosis of infection can be difficult in intensive care patients but is usually easier in less ill patients with a central venous catheter. Quantitative or semiquantitative laboratory techniques can be used to confirm the diagnosis in the appropriate clinical setting. A variety of preventive measures have been shown to minimize the risk of development of catheter-related bloodstream infection, including use of maximal aseptic technique for insertion, use of special teams for care of the catheter, limiting manipulation of the catheter, use of povidoneiodine
ointment and cotton gauze dressings for recently inserted catheters, a silver-impregnated collagen cuff and antiseptic-impregnated catheters [60] [61] [62].

Central venous catheters (CVCs) are widely used in critically ill patients in intensive care units. However, infectious complications are common and may limit their utility. The literature was reviewed to determine the impact of CVC design and composition, insertion site selection, insertion procedures, care and removal of temporary CVCs on infectious complications. Relevant articles were identified and selected for review using a database search (Medline and manual of the English language literature) based upon study design and sample size with an emphasis on prospective randomized trials. To minimize infectious complications and maintain a reasonable cost-benefit ratio, it was recommended: i) use a single lumen catheter unless clear indications for a multi-lumen catheter exist; ii) insert the catheter via the subclavian vein if no relative contraindication exists (bleeding diathesis, positive pressure ventilation); iii) disinfect the insertion site employing sterile technique; iv) apply a dry, sterile dressing and change the dressing every other day; v) inspect the insertion site for signs of infection and remove the catheter if pus is present; vi) if a catheter-related infection is suspected, change the catheter over a guidewire and culture the distal segment. The replacement catheter should be removed if an original catheter segment culture is positive [63].

1.3.3 Other biomedical applications (wound dressings, pins ect.)

Infections with multiresistant bacteria have become a serious problem in joint arthroplasty. A study report [64] about in vitro antibacterial activity against multiresistant bacteria and in vitro cytotoxicity of polymethylmethacrylate bone
cement loaded with metallic silver particles with a size of 5-50 nm called NanoSilver. In vitro antibacterial activity against *S. epidermidis*, methicillin-resistant *S. epidermidis* (MRSE), and methicillin-resistant *S. aureus* (MRSA) was studied by microplate proliferation tests. Quantitative elution testing and qualitative ongrowth of human osteoblasts was done to study in vitro cytotoxicity. Only NanoSilver cement showed high-antibacterial activity against all strains, including MRSE and MRSA. Gentamicin cement was not effective against MRSA and MRSE due to the high-level gentamicin resistance of the tested strains. Plain cement did not inhibit proliferation of any strains. There was no significant difference regarding in vitro cytotoxicity between NanoSilver and the non-toxic control. Cytotoxicity of cement loaded with silver salts made this kind of silver unsuitable for all day clinical used in the past. This new form of silver called NanoSilver was free of in vitro cytotoxicity and showed high effectiveness against multiresistant bacteria. If the results can be confirmed in vivo, NanoSilver may have a high interest in joint arthroplasty [64].

The most significant complication in external fixation is pin tract infection causally related to the highly adaptive ability of bacteria to colonise the surfaces of "inert" biomaterials or of adjacent damaged tissue cells. The hypothesis that coating a pin with a silver-containing compound will decrease bacterial colonisation and/or pin tract infection has been confirmed in other studies in vitro and in vivo experiments. In some work, biocompatibility of silver-coated orthopaedic external fixation pins was compared with stainless steel controls in an in vitro study. Human peripheral blood lymphocytes were used to assess the possible genotoxic effect of silver, studying the frequency of sister-chromatid exchanges and micronuclei while fibroblasts (NIH 3T3) and osteoblast-like cells
were used for cytotoxicity and cytocompatibility studies. These studies have shown that silver is neither genotoxic nor cytotoxic compared to stainless steel, a material in wide use as a metal implant. At 4 days cells cultured on the silver-coated material evidenced good cell spreading and a higher cell count with respect to the uncoated material. It appears that the addition of silver onto implantable medical devices could be beneficial when specific biological properties, such as antibacterial behaviour, are required. Based on these and the previous bacterial studies it seems like the toxicity towards bacteria was significantly larger than that towards the human cells [65].

1.4 Mechanisms of silver anti-bacteria action

Infections are often caused by bacteria that gain access either from the skin during insertion, or by migration from the skin to the surrounding subcutaneous tissue, once in situ. It is necessary to understand the pathogenesis with regard to sessile or planktonic forms of microorganisms and the reason why they adhere to catheter surfaces, and the interactions between host, catheter and bacteria. To prevent colonization two general approaches have been adopted: the first concerns the site of insertion--prophylactic antibiotics, tunnelling, intraluminal antibiotic locks, topical application of antibiotics or antiseptics, cutaneous disinfection pre- and post-insertion with various antiseptics, catheter site care and frequency of dressing changes. The second approach considers the physical nature of catheter material--silver impregnated subcutaneous collagen cuffs, antiseptic hubs, antimicrobial coating of catheters with antibiotics e.g., rifampicin and minocycline, silver ions, chlorhexidine, or chlorhexidine and silver sulphadiazine. Only by understanding the pathogenesis of catheter devices whereby microorganisms are embedded in
biofilms and are resistant to the action of antibiotics, will effective preventative measures be possible [66].

It is not well known why silver has a high antibacterial activity to microorganisms. It is thought that this antibacterial activity is caused by adsorption of silver into the microorganisms [28].

The relationship between the reduction of silver and antibacterial activity was observed by using an electrochemical approach. The electrochemical method was to control the oxidation state of silver by application of an external voltage. The antibacterial activity did not occur at the change of the oxidation state of silver from +1 to 0. The antibacterial activity occurred at the cyclic redox reaction between silver ion and silver metal. The results indicated that it was not the reduction reaction but instead the oxidation reaction was necessary for antibacterial activity. And during this process dissolved oxygen would be reduced at the oxidation of silver [28].

In fact, attempts to use silver and electric current for therapeutic purposes go back many years. The American Brown in 1879 tried to stimulate healing on a patient with burns which were not responding to therapy with the aid of a silver plate placed on the wound and connected via a silver wire to a copper plate which was fastened on a piece of wet leather placed on the healthy skin [67]. No attention was paid to a possible effect on microorganisms at this time [68].

Schmalzel et al. and Chu et al. also studied the effect of electric current on silver-containing materials [69,70]. They treated experimental animals whose burns had been inoculated with a lethal dose of *P. aeruginosa* with silver metal-coated nylon cloth. An electric circuit was created by inserting a silver needle under the burn wound and connecting the needle and the silver dressing via an electrode. A
regulated constant current was delivered by a power supply. The maximum voltage varied between 0.1 and 0.86 V at 0.4±40 mA. The current was applied for 5 days. If the silver nylon was used as a surface anode and the silver needle as cathode, a therapeutic antibacterial effect was produced. It was found that silver nylon without current had a much lower antibacterial effect than silver nylon with current. Subsequent investigations of silver nylon with weak direct current concentrated on the positive influence on wound healing in experimental animals [71-73].

Some work investigated the biological mechanism of silver action. It has been suggested that its anti-bacterial action arises from the formation of silver ions (Ag+) which bind strongly to electron donor groups such as sulphur, oxygen and nitrogen in large molecules of biological importance [54]. This in turn results in the displacement of other metal ions essential to the maintenance of a healthy bacterial cell including Ca^{2+} and Zn^{2+}. The antibacterial kill rate of silver ions is directly proportional to silver ion concentration.

1.5 Toxicity of silver

Ionic silver exhibits antimicrobial activity against a broad range of microorganisms. As a consequence, silver is included in many commercially available healthcare products. The use of silver is increasing rapidly in the field of wound care, and a wide variety of silver-containing dressings are now commonplace (e.g. Hydrofiber dressing, polyurethane foams and gauzes). However, concerns associated with the overuse of silver and the consequent emergence of bacterial resistance are raised. The current understanding of the biochemical and molecular basis behind silver resistance has been documented since 1998. Despite the
sporadic evidence of bacterial resistance to silver, there have been very few studies undertaken and documented to ascertain its prevalence. The risks of antibacterial resistance developing from the use of biocides may well have been overstated. It is proposed that hygiene should be emphasized and targeted towards those applications that have demonstrable benefits in wound care. Liedberg et al. reviewed some literature to assess the likelihood of widespread resistance to silver and the potential for silver to induce cross-resistance to antibiotics, in light of its increasing usage within the healthcare setting [74].

Antimicrobial activity of silver is due to its complexation to membranes, enzymes, nucleic acids, and other cellular components. Silver ions complex strongly to electron donor groups containing nitrogen, oxygen, and sulfur, which are present in microbial cells as amines, hydroxyls, phosphates, and thiols. As with other heavy metals, silver is known to react readily with sulfur-containing compounds. It was postulated that the thiol group-containing enzymes such as lactate dehydrogenase were inhibited by the binding of Ag^+, resulting in conformational change. It has been postulated that silver exerts its toxicity at multiple sites, resulting in leakage of cellular metabolites. Potassium is one of the intracellular ions that leaks due to Ag^+ toxicity. Indirect toxicity may arise from salt formation with silver ions that result in a chloride or anion limitation to the cell. Bacterial consortia that exhibit an adaptive tolerance to silver have been isolated. It is also postulated that silver may compete for cellular entry by an essential copper transport system. Intracellular metal accumulation offers a greater uptake capacity than simple silver binding to the cell surface. Once taken up, silver may bind to various cellular structures. Silver is known to stain proteins and has been used after gel electrophoresis to visualize protein bands. It was found
that an SSB-1 RNA-binding protein in yeast was demonstrated to be a nucleolar-specific silver-binding protein in organism. In addition to proteins, silver has been found to bind nucleic acids. The binding of silver while offering advantages for cellular uptake also raises serious concern about the ultimate fate of such particles or ions in the body with protein binding offering the material access to a number of physiological compartments which otherwise would be inaccessible. Evidence for uptake and accumulation of silver suggests a close association of the abilities with silver resistance and detoxification. It appears likely that resistant cells exclude or efflux silver unless some type of intracellular detoxification mechanism (such as metallothionein) exists. The Ag⁺ resistance pKK1 plasmid (49.4 MDa) which was first described by Slawson et al. was isolated from *P. stutzeri* and introduced into *P. putida* by high-voltage electrotransformation. It was found that the *P. putida* transformant was resistant to AgNO₃ [75].

The toxicity of silver-coated urinary catheters was assessed using a cell culture technique. The inhibitory effect of catheter extracts on the uptake of 3H-labelled thymidine by mouse fibroblasts was measured. The results show that silver coating had no toxic effect whereas silver nitrate and silver sulphate coating did have a toxic effect [76].

### 1.6 Introduction to current project
1.6.1 Project proposal

In recent years, silver has gained in importance as an antimicrobial agent through incorporation in coatings applied to wound dressings, urinary catheters and other materials which are required to prevent the onset of infection. Silver has also been used in water treatment (sometimes in conjunction with copper) and other sterilization applications in the food processing and packaging industries. However, too much silver ingested over an extended period of time (sometimes from ingestion of colloidal silver system) can result in a condition known as argyria causing skin to turn an irreversible grey-blue colour. Thus, it is important to carefully control the amount of silver administered to the human body.

The essential aim of this project is to study the mechanism of silver dissolution from silver and silver-platinum alloys, and their polymeric coatings. The understanding of silver dissolution in saline solution may provide information for the development of suitable hygienic coatings for medical devices. Electrochemical methods (eg. polarization curves) are applied to understand and to predict the corrosion behaviour of silver metals and alloys in the presence of saline solutions [77].

The influence of coating composition, environment and surface characteristics will be determined. Especially important is the identification of the role of platinum in enhancing silver dissolution from silver-platinum alloys and its influence on surface film reduction. Fundamental oxidation/reduction chemistry of polymer materials incorporating silver will be studied as well.

This information will considerably enhance the future design and performance of silver-releasing coatings resulting in improved efficacy of biomedical device
surfaces and in the relatively new field of hygienic coatings in areas requiring extremely high levels of cleanliness. Ultimately this will benefit the burgeoning Irish biomedical device and food production/packaging industries through future collaborative industrial research.

Previous work [25,26] studied a range of silver-platinum alloys and the coatings of silver and silver-platinum alloys and their antibacterial activity. They found that platinum did enhance the release of silver ions from the coating of 1\%Pt-Ag alloy coated on silicon. This project will continue to study the effects of different coatings (Ag/Pt alloy deposited by sputter coating and silver contained polymers) and the effects of the conditions (such as the electrolyte, temperature, concentration of O$_2$) on the release of silver ions that are able to kill bacteria and at the same time to avoid cytotoxicity.

1.6.2 Aims of the project

The aim is to increase an understanding of the relationship between electrochemistry and corrosion, the expression of the corrosion rate, the types of corrosion, and the effect factors on the corrosion (temperature, electrolyte, pH value, O$_2$ level, and the concentration of other ions in the solution).

In order to enhance the anti-bacterial performance of silver in such coatings, it is useful to promote Ag$^+$ formation relative to that of metallic silver itself. The aims of this work are as follows:
1. With a view to enhancing the dissolution, silver and silver-platinum alloys will be used to determine whether the inclusion of platinum enhances silver dissolution.

2. Two approaches of the coatings on polymers will be undertaken.

   a. Thin metallic films of silver or silver-platinum alloys will be sputter coated on plastics, typically used for catheters.

   b. Silver nanoparticle polymeric composites will be prepared and cast with a view to gauge silver delivery.

3. The antibacterial activity and the cytotoxicity of silver nanoparticle polymeric composites will be measured.
Chapter 1 Introduction

1.7 References

Chapter 1 Introduction


Chapter 1 Introduction


Chapter 2 Experimental Techniques and Related Theories
Chapter 2 Experimental Techniques and Related Theories

Abstract

This chapter gives a general introduction to the principles of the electrochemical methods, and techniques used for characterization and identification of a material. Cyclic Voltammetry (CV), Zero Resistance Amperometry (ZRA), and Electrochemical Impedance Spectroscopy (EIS) techniques were applied to investigate silver dissolution behaviour and the effect of platinum on silver dissolution behaviour.

To characterize polymeric silver nano-particle composites prepared in this work, UV vis spectroscopy, X-ray Diffraction (XRD), Transmission electron Microscopy (TEM), Scanning Electron Microscopy (SEM) with Energy Dispersion X-ray (EDX) analysis, Raman Spectroscopy, and Atomic Absorption (AA) analysis were employed.
2.1 Introduction

This work studies the mechanism of silver dissolution from silver containing materials for biomedical and hygienic coating applications. The material containing silver will be studied in a biological environment. A new research subject of bioelectrochemistry has been developed for studying the electrochemistry of proteins, enzymes, and cellular components. Similarly, this work employs some electrochemical methods to achieve an understanding of the mechanism of silver dissolution from the biomaterials.

The electrochemical methods to be applied in this work include Cyclic Voltammetry (CV) technique, Zero Resistance Amperometry (ZRA) measurement, Electrochemical Impedance Spectroscopy (EIS) to study silver dissolution behaviour and the effect of platinum on silver dissolution behaviour from silver platinum alloys and polymeric material containing silver or silver-platinum alloys. Cyclic voltammetry was performed by using the Solartron SI 1287/1255B potentiostat instrumentation. For example, CV studies the reactions such as film reduction, film formation, silver dissolution ($\text{Ag}^+$) and monolayer deposition in some potential region take place in the silver (silver-platinum) material in the physiological solutions. The basic principles of these technique and applications will be described in the following part of this chapter [11,2-4].

Polymeric silver nano-particle composites were prepared and characterized by UV vis spectroscopy, XRD, SEM, TEM and Raman Spectroscopy.
2.2 Electrochemical technique and related theories

An electrode is a conductor that donates or collects electrons in a cell. The electrode that has an electron and is positive is the anode, where oxidation occurs. The cathode is the negative electrode, where reduction occurs and electrons are collected.

An electrode that serves as a transducer responding to the excitation signal and the concentration of the substance of interest in the solution being investigated, and that permits the flow of current sufficiently large to effect appreciable changes of bulk composition within the ordinary duration of a measurement is called working electrode (WE). The working electrode should be able to characterize the material in terms of its chemical composition and metallurgical condition. The surface treatment of the working electrode is also important. This should be reproducible and relevant to the intended application. Typical surface conditions include as received (this is often very relevant, but not very reproducible), or the electrode may be ground and polished. Cut edges will often behave differently from the surface and should be avoided if possible (unless it is intended to study the behaviour of the edge). Electrochemical reactions are very sensitive to surface contamination, and it is important to clean the specimen before testing, particularly to remove grease. However, care must also be taken to avoid contamination of the surface with species extracted from mounting materials (e.g. epoxy resins) by powerful solvents such as acetone.

A reference electrode is used in measuring the working electrode potential of an electrochemical cell. A reference electrode should have a stable electrochemical potential as long as no current flows through it.
Reference electrodes are composed of four distinct parts, an inert outer body, a reference element, an electrolyte(s), and a liquid junction(s). The body acts as a reservoir for the reference electrolyte and provides protection for the glass electrode, as in the case of a combination electrode.

The most common laboratory reference electrodes are the Saturated Calomel Electrode (SCE) and the Silver/Silver Chloride (Ag/AgCl) electrodes. A Luggin capillary is often used to position the sensing point of a reference electrode to a desired point in a cell.

SCE and Ag/AgCl reference electrodes are employed in this work.

The Counter Electrode (or Secondary or Auxiliary Electrode) must be inert in the conditions to which it is exposed in order to avoid contamination of the solution. Typically platinum or graphite is used as counter electrode. It is often specified that the counter electrode should have a large area compared to the working electrode, but this may not be necessary in many cases. The fundamental requirement is that it will be able to pass sufficient current into the solution without needing an excessive cell voltage or creating a non-uniform current distribution on the working electrode. A platinum foil was chosen as the counter electrode in this work.

The electrolyte is the medium between the electrodes in the cell, and it consists of the solvent and a high concentration of an ionised salt as well as the electroactive species. It may also contain other materials, complexing agents, buffers.

An electrochemical cell consists of a pair of electrodes immersed in solutions of electrolytes and connected via an external metallic conductor. If the cell is used as a source of electrical energy, (i.e. if it converts the free energy of a physical or chemical change into electrical free energy), it is called a galvanic cell or a voltaic
cell. Figure 2.1 shows a sample of a galvanic cell that consists the electrodes of plates of Zn and Cu in solutions of ZnSO₄ and CuSO₄ as a Cu-Zn battery [21].

![Figure 2.1 an example of a Cu-Zn Galvanic cell [21]](image)

If the cell is used to achieve a change in the physical or chemical composition of the constituents of the cell by the application of electrical energy from some external source, it is called an electrolytic cell. A typical electrochemical cell consisting of three electrodes: a working electrode, a counter electrode and a reference electrode.

The potentiostat is a device for controlling the potential between RE and WE while allowing current to flow to the CE. The simplest setup, which is of little practical use but outlines the principle, is shown in Figure 2.2. It can be seen that the device is simply a voltage follower maintaining the output voltage between RE and WE at the programming potential Eᵋ. WE has a potential Eᵋ relative to RE, so that the input voltage is inverted in the cell. Also in this arrangement there is no device for measuring the current through the cell and the potentiostat is unable to apply more than a single potential at any one time.
2.2.1 Cyclic Voltammetry technique

2.2.1.1 Theory of Cyclic Voltammetry

Cyclic voltammetry is the most widely used technique for acquiring qualitative information about electrochemical reactions. The power of cyclic voltammetry results from its ability to rapidly provide considerable information on the thermodynamics of redox processes and the kinetics of heterogeneous electron-transfer reactions, and on coupled chemical reactions or adsorption processes. Cyclic voltammetry is often the first experiment performed in an electro-analytical study. In particular, it offers a rapid location of redox potentials of the electro-active species, and convenient evaluation of the effect of media upon the redox process.
CV consists of scanning linearly the potential of a stationary working electrode, using a triangular potential waveform. The electrode potential is ramped linearly to a more negative potential, and then ramped in reverse back to the starting voltage. The forward scan produces a current peak for any analyte that can be reduced through the range of the potential scan. The current will increase as the potential reaches the reduction potential of the analyte, but then falls off as the concentration of the analyte is depleted close to the electrode surface. As the applied potential is reversed, it will reach a potential that will reoxidize the product formed in the first reduction reaction, and produce a current of reverse polarity from the forward scan.

The cyclic voltammogram is characterized by several important parameters. Four of these observables, the two peak currents and two peak potentials, provide the basis for the diagnostics developed by Bard and Faulkner [5] for analyzing the cyclic voltammetric experiment.

The peak current for a reversible couple at 25°C is given by the Randles-Sevcik equation [5]:

\[
i_p = (2.69 \times 10^5) n^{3/2} A C D^{1/2} v^{1/2}
\]  

(2-1)

where \( n \) is the number of electrons, \( A \) is the electrode area (cm\(^2\)), \( C \) is the concentration (mol/cm\(^3\)), \( D \) is the diffusion coefficient (cm\(^2\)/s), and \( v \) is the scan rate (V/s). Accordingly, the current is directly proportional to concentration and increases with the square root of the scan rate. The ratio of the reverse-to-forward peak currents \( (i_{pr}/i_{pf}) \) is unity for a simple reversible couple. This peak ratio can be strongly affected by chemical reactions coupled to the redox process. The current peaks are commonly measured by extrapolating the preceding baseline current.

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The position of the peaks on the potential axis ($E_p$) is related to the formal potential of the redox process. The formal potential for a reversible couple is centered between $E_{pa}$ and $E_{pc}$:

$$E^\circ = (E_{pa} + E_{pc})/2 \quad (2-2)$$

The separation between the peak potentials (for a reversible couple) is given by:

$$\Delta E_p = E_{pa} - E_{pc} = 57\text{mV}/n$$

Thus, the peak separation can be used to determine the number of electrons transferred, and as a criterion for a Nernstian behavior. Accordingly, a fast one-electron process exhibits a $\Delta E_p$ of about 57 mV. Both the oxidation and reduction peak potentials are independent of the scan rate. It is possible to relate the half-peak potential ($E_{p/2}$, where the current is half of the peak current) to the polar graphic half-wave potential, $E_{1/2}$

$$E_{p/2} = E_{1/2} \pm 29\text{mV}/n \quad (2-3)$$

The sign is positive for a reduction process. For multielectron-transfer reversible processes, the cyclic voltammogram consists of several distinct peaks, if the $E^\circ$ values for the individual steps are successively higher and are well separated.

The potential difference between the reduction and oxidation peaks is theoretically 57 mV for a reversible reaction. In practice, the difference is typically 70-100 mV. Larger differences, or nonsymmetric reduction and oxidation peaks are an indication of a nonreversible reaction. These parameters of cyclic voltammograms make CV most suitable for characterization and mechanistic studies of redox reactions at electrodes.

Although cyclic voltammetry is very widely used for the initial redox characterization of a molecule (i.e., the redox potentials, and the stability of the
different oxidations states) and for qualitative investigation of chemical reactions that accompany electron transfer, there are a number of disadvantages inherent in this technique:

a. It cannot be separated. If both of these effects are present, then the rate constants for these processes can only be calculated using simulation methods.

b. There is a background charging current throughout the experiment of magnitude $n C_{dl}$ (where $C_{dl}$ is the capacitance of the interface at the working electrode). This restricts the analytical detection limit to about $10^{-5}$ M. In addition, the ratio of the peak faradaic current to the charging current decreases with increasing $n$ (since $i_p$ is proportional to $v^{1/2}$), and this places an upper limit on the value of $v$ that can be used.

In spite of these limitations, cyclic voltammetry is very well suited for a wide range of applications. Indeed, in some areas of research, cyclic voltammetry is one of the standard techniques used for characterization.

### 2.2.1.2 Calibration of the experimental conditions on CV test

A CV experiment was carried out for studying the reversible reaction of $Fe^{2+} - e^- = Fe^{3+}$ before the Ag and Ag-Pt alloy discs were studied by CV.

**Equipment:** a Solartron 1287 potentiostat

**Electrode:** WE, Pt wire; RE: SCE; CE, Pt Wire.

**Procedure:**

1. Weigh 0.74 g KCl to make 0.1M 100mL KCl solution;
2. Take 50 mL KCl solution and put into a beaker contained 0.1055 g $K_4Fe(CN)_6\cdot 3H_2O$ to make 0.005M $K_4Fe(CN)_6$/KCl solution;
3. Connect the electrodes of (WE, RE and CE) with the Potentiostat/Galvanostat instrument and put these electrodes into the beaker to make a cell;

4. Place the cell into Faraday cage;

5. Turn on the instruments and the computer;

6. Collect data by CorrWare programs.

Figure 2.3 Cyclic voltammograms of the \([\text{Fe(CN)}_6]^{3-/4-}\) couple at platinum. The concentration of the couple is 0.005M and the background electrolyte is 0.1M KCl, scan rate: 10mV/s
The CV result of the reversible reaction of $\text{Fe}^{2+} - \text{e}^- = \text{Fe}^{3+}$ collected at a scan rate of $10 \text{ mV/s}$ is shown in Figure 2.3. This indicated that the condition of the Solartron potentiostat was in good calibration.

2.2.1.3 The experimental set-up for CV test

Figure 2.4 Experimental set-up for cyclic voltammetric studies on silver and silver-platinum alloys
A special cylindrical shaped electrochemical cell (EG&G PARC Flat Cell) was used to clamp a silver-platinum target to one end, which served as a working electrode in a normal three-electrode configuration (Figure 2.4). In this system a Teflon ring seal leaves exposed a test electrode area of 0.785cm$^2$. The other two electrodes employed in this work were a platinum foil as the counter (auxiliary) electrode and a saturated calomel reference electrode (SCE) as the reference electrode. Potential values quoted in this work are referred to this electrode that has a value of 242 mV vs. the standard hydrogen electrode.

In this work, Ag and Ag-Pt alloy sputter targets were specially commercialised from Testbourne with platinum levels of 0.5%, 1.5%, 3% and 5% by weight. They are employed as the working electrode.

For the details of the conditions each experiment will be described in each chapter.

### 2.2.2 Zero Resistance Amperometry

A standard current meter is usually built in typical multimeters to measure the current as a voltage drop across a shunt resistor. A usual digital 3 ½ digit - voltmeter has a lowest current range of 200 mV, accordingly rises the voltage drop across the internal shunt resistor up to 200 mV when the current corresponds to the full range.

When measuring the current in usual electronic circuits, this voltage drop may be disregarded. Concerning electrochemical potentials, a drop of 100 mV is significant, for example, Tafel slopes at ambient temperature may vary by 29 mV
per current decade. This requires that an ammeter for electrochemical measurements should ideally have zero internal resistance.

To achieve this, a special zero resistance ammeter, also called active current sinks (CS), is used. Any potentiostat can be used also as such an active current sink, and it is very simple to do so. Connect the counter electrode to the reference electrode, and use the internal range resistor to define the current range. The current is now directly displayed on the built-in current meter, and a corresponding voltage is fed to the current output terminal.

Figure 2.5 Potentiostat configured to act as a Zero Resistance Amperometer

The polarity of the current that passes the working electrode is positive if the meter deflects to the right (positive) side. If the internal range resistor does not give the required resolution, you can introduce an external one (Figure 2.5). Then the current is measured as the voltages between CE terminal and ground according to Ohm’s law:
where \( R \) is the external range resistor. For \( R = 1 \text{ MOhm} \), \( I = \frac{E}{R} = 1 \text{ V}/\mu\text{A} \). A capacitor across the external range resistor will be useful to reduce noise. The time constant of \( R \times C \) should be in the order of ms for currents down to 1 µA, for lower currents \( R \times C \) should be in the order of 100 ms up to 1 s.

This work used ZRA measurement to investigate the galvanic reaction taking place between silver or silver-platinum alloys and platinum. Further discussion was shown in Chapter 3.

2.2.3 Electrochemical Impedance Spectroscopy (EIS)

2.2.3.1 Theories related to EIS

Electrochemical Impedance is normally measured using a small excitation signal. This is done so that the cell's response is pseudo-linear. In a linear (or pseudo-linear) system, the current response to a sinusoidal potential will be a sinusoid at the same frequency but shifted in phase (Figure 2.6)
The excitation signal, expressed as a function of time, has the form

\[ E(t) = E_0 \cos(\omega t) \quad (2-5) \]

where \( E(t) \) is the potential at time \( t \), \( E_0 \) is the amplitude of the signal, and \( \omega \) is the radial frequency. The relationship between radial frequency \( \omega \) (expressed in radians/second) and frequency \( f \) (expressed in hertz) is:

\[ \omega = 2\pi f \quad (2-6) \]

In a linear system, the response signal, \( I_0 \), is shifted in phase and has a different amplitude, \( I_0 \):

\[ I(t) = I_0 \cos(\omega t - \phi) \quad (2-7) \]

An expression analogous to Ohm's Law calculates the impedance of the system as:

\[ Z = \frac{E(t)}{I(t)} = \frac{E_0 \cos(\omega t)}{I_0 \cos(\omega t - \phi)} = Z_0 \frac{\cos(\omega t)}{\cos(\omega t - \phi)} \quad (2-8) \]
The impedance is therefore expressed in terms of a magnitude, $Z_0$, and a phase shift, $\phi$. When the applied sinusoidal signal is plotted on the X-axis of a graph and the sinusoidal response signal $I(t)$ on the Y-axis, an oval is plotted (Figure 2.7). This oval is known as a "Lissajous figure". Analysis of Lissajous figures on oscilloscope screens was the accepted method of impedance measurement prior to the availability of lock-in amplifiers and frequency response analyzers.

![Figure 2.7 Lissajous figure](image)

Using Eulers relationship,

$$\exp(j\phi) = \cos\phi + j\sin\phi \quad (2-9)$$

It is possible to express the impedance as a complex function. The potential is described as,

$$E(t) = E_0 \exp(j\omega t) \quad (2-10)$$

and the current response as,

$$I(t) = I_0 \exp(j\omega t - j\phi) \quad (2-11)$$
The impedance is then represented as a complex number,

\[ Z = \frac{E}{I} = Z_0 \exp(j\phi) = Z_0 (\cos\phi + j\sin\phi) \quad (2-12) \]

The expression for \( Z(\omega) \) is composed of a real and an imaginary part. If the real part is plotted on the X axis and the imaginary part on the Y axis of a chart, a "Nyquist plot" is shown in Figure 2.8. Notice that in this plot the y-axis is negative and that each point on the Nyquist plot is the impedance at one frequency.

![Nyquist plot with impedance vector](image)

**Figure 2.8 Nyquist plot with impedance vector**

On the Nyquist plot the impedance can be represented as a vector of length \(|Z|\). The angle between this vector and the x-axis is \( \phi \).

Another popular presentation method is the "Bode plot". The impedance is plotted with log frequency on the x-axis and both the absolute value of the impedance (\(|Z| = Z_0\)) and phase-shift on the y-axis. Unlike the Nyquist plot, the Bode plot explicitly shows frequency information (Figure 2.9).
2.2.3.2 Physical Electrochemistry and Equivalent Circuit Elements

a. Electrolyte Resistance

Solution resistance is often a significant factor in the impedance of an electrochemical cell. A modern 3-electrode potentiostat compensates for the solution resistance between the counter and reference electrodes. However, any solution resistance between the reference electrode and the working electrode must be considered when a cell is to be modeled.

The resistance of an ionic solution depends on the ionic concentration, type of ions, temperature and the geometrical area in which current is carried. In a bounded area with area $A$ and length $l$ carrying a uniform current the resistance is defined as:

\[
\text{Resistance} = \frac{\sigma A}{l}
\]
\[ R = \rho \frac{1}{A} \quad (2-13) \]

where \( R \) is the solution resistance. The resistivity of the solution, \( \rho \), is more commonly used in solution resistance calculations.

Most electrochemical cells do not have uniform current distribution through a definite electrolyte area. The major problem in calculating solution resistance therefore concerns determination of the current flow path and the geometry of the electrolyte that carries the current. This can be easily solved through fitting a model to experimental EIS data without calculating solution resistance from ionic conductance.

b. Double Layer Capacitance

An electrical double layer exists at the interface between an electrode and its surrounding electrolyte. This double layer is formed as ions from the solution adsorbed on the electrode surface. Charges in the electrode are separated from the charges of these ions. The separation is very small, on the order of angstroms. Charges separated by an insulator form a capacitor. On a bare metal immersed in an electrolyte, it can be approximately estimated as 30 \( \mu \text{F} \) of capacitance for every cm\(^2\) of electrode area [5,6]. The value of the double layer capacitance depends on many variables including electrode potential, temperature, ionic concentrations, types of ions, oxide layers, electrode roughness, impurity adsorption.

c. Polarization Resistance

Whenever the potential of an electrode is forced away from its value at open circuit that is referred to as polarizing the electrode. When an electrode is polarized, it can cause current to flow via electrochemical reactions that occur at the electrode surface. The amount of current is controlled by the kinetics of the
reactions and the diffusion of reactants both towards and away from the electrode. In cells where an electrode undergoes uniform corrosion at open circuit, the open circuit potential is controlled by the equilibrium between two different electrochemical reactions. One of the reactions generates cathodic current and the other anodic current. The open circuit potential ends up at the potential where the cathodic and anodic currents are equal. It is referred to as a mixed potential. The value of the current for either of the reactions is known as the corrosion current. Mixed potential control also occurs in cells where the electrode is not corroding. When there are two simple, kinetically controlled reactions occurring, the potential of the cell is related to the current by the following expression [7,8]:

\[
I = I_{\text{corr}} \left(10^{\frac{(E - E_{oc})}{\beta_a}} - 10^{\frac{(E - E_{oc})}{\beta_c}}\right) \tag{2-14}
\]

where

- \(I\) is the measured cell current in amps,
- \(I_{\text{corr}}\) is the corrosion current in amps,
- \(E_{oc}\) is the open circuit potential in volts,
- \(\beta_a\) is the anodic Beta coefficient in volts/decade,
- \(\beta_c\) is the cathodic Beta coefficient in volts/decade.

If we apply a small signal approximation (E-Eoc is small) to equation 2-14, we get the following [7,8]:

\[
I_{\text{corr}} = \frac{\beta_a \beta_c}{2.303 (\beta_a + \beta_c)} \left(\frac{1}{R_p}\right) \tag{2-15}
\]

which introduces a new parameter, \(R_p\), the polarization resistance. The polarization resistance behaves like a resistor.
If the Tafel constants are known, $I_{\text{corr}}$ can be calculated from $R_p$ using equation 2-15. $I_{\text{corr}}$ in turn can be used to calculate a corrosion rate.

d. Charge Transfer Resistance

A similar resistance is formed by a single kinetically controlled electrochemical reaction. Consider a metal substrate in contact with an electrolyte. The metal molecules can electrolytically dissolve into the electrolyte, according to:

$$\text{Me} = \text{Me}^{\alpha^+} + \text{ne}^- \quad (2-16)$$

or more generally:

$$\text{Red} = \text{Ox} + \text{ne}^- \quad (2-17)$$

In the forward reaction in the first equation, electrons enter the metal and metal ions diffuse into the electrolyte. Charge is being transferred.

This charge transfer reaction has a certain speed. The speed depends on the kind of reaction, the temperature, the concentration of the reaction products and the potential.

The general relation between the potential and the current for a kinetically limited process is:

$$i = i_o \left[ \frac{C_0}{C_0^*} e^{\frac{-\alpha n F \eta}{RT}} - \frac{C_0^*}{C_R} e^{\frac{(1-\alpha) n F \eta}{RT}} \right] \quad (2-18)$$

with,

- $i_o = \text{exchange current density}$
- $C_o = \text{concentration of oxidized species at the electrode surface}$
- $C_0^* = \text{concentration of oxidized species in the bulk}$
- $C_R = \text{concentration of reduced species at the electrode surface}$
- $F = \text{Faraday's constant}$
- $T = \text{temperature}$
- $R = \text{gas constant}$
- $\alpha = \text{transfer coefficient}$
- $n = \text{number of electrons involved}$
- $\eta = \text{overpotential (E - E_0)}$
The overpotential, $\eta$, measures the degree of polarization. It is the electrode potential minus the equilibrium potential for the reaction.

When the concentration in the bulk is the same as at the electrode surface, $C_0=C_0^*$ and $C_R=C_R^*$. This simplifies equation 2-18 into:

$$i = i_0 \left[ e^{-\frac{nF\eta}{RT}} - e^{-\frac{(1-\alpha)nF\eta}{RT}} \right]$$  \hspace{1cm} (2-19)

This equation is called the Butler-Volmer equation. It is applicable when the current depends only on the charge transfer kinetics.

Stirring will minimize diffusion effects and keep the assumptions of $C_0=C_0^*$ and $C_R=C_R^*$ valid.

When the overpotential, $\eta$, is very small and the electrochemical system is at equilibrium, the expression for the charge transfer resistance changes into:

$$R_{ct} = \frac{RT}{nF_i}$$  \hspace{1cm} (2-20)

From this equation the exchange current density can be calculated when $R_{ct}$ is known.

e. Diffusion

Diffusion can create an impedance known as the Warburg impedance. This impedance depends on the frequency of the potential perturbation. At high frequencies the Warburg impedance is small since diffusing reactants don't have to move very far. At low frequencies the reactants have to diffuse farther, thereby increasing the Warburg impedance.

The equation for the infinite Warburg impedance is [8]:

$$Z = \sigma (\omega)^{1/2} (1 - j)$$  \hspace{1cm} (2-21)
On a Nyquist plot the infinite Warburg impedance appears as a diagonal line with a slope of 0.5. On a Bode plot, the Warburg impedance exhibits a phase shift of 45°.

In equation 2-21, \( \sigma \) is the Warburg coefficient defined as [8]:

\[
\sigma = \frac{R T}{n^2 F^2 A \sqrt{2}} \left( \frac{1}{C_0^* \sqrt{D_0}} + \frac{1}{C_R^* \sqrt{D_R}} \right) \quad (2-22)
\]

In which,
- \( \omega \) = radial frequency
- \( D_0 \) = diffusion coefficient of the oxidized species
- \( D_R \) = diffusion coefficient of the reduced species
- \( A \) = surface area of the electrode
- \( n \) = number of electrons transferred
- \( C^* \) = bulk concentration of the diffusing species (moles/cm\(^3\))

This form of the Warburg impedance is only valid if the diffusion layer has an infinite thickness. Quite often this is not the case. If the diffusion layer is bounded, the impedance at lower frequencies no longer obeys the equation above. Instead, it is:

\[
Z_0 = \sigma \omega^{1/2} (1 - j) \tan \left( \frac{j \omega}{D} \delta \right)^{1/2} \quad (2-23)
\]

with,
- \( \delta \) = Nernst diffusion layer thickness
- \( D \) = an average value of the diffusion coefficients of the diffusing species

This more general equation is called the finite Warburg impedance.

f. Coating Capacitance

A capacitor is formed when two conducting plates are separated by a non-conducting media, called the dielectric. The value of the capacitance depends on the size of the plates, the distance between the plates and the properties of the dielectric. The relationship is:
Whereas the electrical permittivity is a physical constant, the relative electrical permittivity depends on the material.

Notice the large difference between the electrical permittivity of water and that of an organic coating. The capacitance of a coated substrate changes as it absorbs water. EIS can be used to measure that change.

g. Constant Phase Element

Capacitors in EIS experiments often do not behave ideally. Instead, they act like a constant phase element (CPE) as defined below.

The impedance of a capacitor has the form:

\[ Z = A (j \omega)^{-\alpha} \]  

When this equation describes a capacitor, the constant \( A = 1/C \) (the inverse of the capacitance) and the exponent \( \alpha = 1 \). For a constant phase element, the exponent \( \alpha \) is less than one.

The "double layer capacitor" on real cells often behaves like a CPE instead of like a capacitor. Several theories have been proposed to account for the non-ideal behavior of the double layer but none has been universally accepted.

h. Virtual Inductor

The impedance of an electrochemical cell can also appear to be inductive which is related to adsorbed reactants. Both the adsorption process and the electrochemical
reaction are potential dependent. The net result of these dependencies can be an inductive phase shift in the cell current.

Inductive behavior can also result from nonhomogeneous current distribution, cell lead inductance and potentiostat non-idealities. In these cases, it represents an error in the EIS measurement.

This work uses a ZPlot software to treat the EIS experimental data to simulate equivalent circuit models that contains a large variety of circuit elements. Some elements are analogs to electrical components such as resistors and capacitors, others have electrical equivalent and are defined by their mathematical functions. The details are further described in Chapter 3.

2.3 Characterization techniques

2.3.1 UV vis Spectroscopy

Ultraviolet visible (UV vis) spectroscopy uses light in the visible and adjacent near ultraviolet (UV) and near infrared (NIR) ranges [9,10]. In this region of the electromagnetic spectrum, molecules undergo electronic transitions. This technique is complementary to fluorescence spectroscopy, in that fluorescence deals with transitions from the excited state to the ground state, while absorption measures transitions from the ground state to a higher energy level state.

A diagram of the components of a typical UV vis spectroscopy is shown Figure 2.10. The functioning of this instrument is relatively straightforward. A beam of light from a visible and/or UV light source (colored red) is separated into its component wavelengths by a prism or diffraction grating. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-
mirrored device. One beam, the sample beam (colored magenta), passes through a small transparent container (cuvette) containing a solution of the compound being studied in a transparent solvent. The other beam, the reference (colored blue), passes through an identical cuvette containing only the solvent. The intensities of these light beams are then measured by electronic detectors and compared. The intensity of the reference beam, which should have suffered little or no light absorption, is defined as $I_0$. The intensity of the sample beam is defined as $I$. Over a short period of time, the spectrometer automatically scans all the component wavelengths in the manner described. The ultraviolet region scanned is normally from 200 to 400 nm, and the visible portion is from 400 to 800 nm.

![Figure 2.10 A diagram of components of a typical UV vis Spectroscopy](image)

A UV vis spectroscopy (UV-210PC MASON Tech.) was employed to characterized the formation of nanoparticles stabilized in polymeric substrates. The results were shown in Chapter 5 and Chapter 6.
2.3.2 X-Ray Diffraction (XRD)

After Roentgen discovered x-rays in 1896, this region of the electromagnetic spectrum provided significant contributions to the fundamental knowledge of atomic structure, and led to the development of techniques for chemical analysis, such as x-ray absorption, x-ray emission, x-ray diffraction (XRD), and x-ray fluorescence (XRF) [11-13].

The atomic planes of a crystal cause an incident beam of X-rays when the wavelength is approximately the magnitude of the interatomic distance to interfere with one another as they leave the crystal. The phenomenon is called X-ray diffraction. It can be applied to analyse materials according to Bragg’s Law (Figure 2.11).

Figure 2.11. The principle of Bragg’s Law

Bragg's law

\[ n \lambda = 2d \sin \theta \]  

(2-26)
where
\[ n = \text{number of wavelengths in the path length} \]
\[ \lambda = \text{the wavelength} \]
\[ d = \text{interatomic distance} \]
\[ \theta = \text{the reflected (and incident) angle} \]

When a primary x-ray excitation source from an x-ray tube or a radioactive source strikes a sample, the x-ray can either be absorbed by the atom or scattered through the material. The process in which an x-ray is absorbed by the atom by transferring all of its energy to an innermost electron is called the "photoelectric effect." During this process, if the primary x-ray has sufficient energy, electrons are ejected from the inner shells, creating vacancies. These vacancies present an unstable condition for the atom. As the atom returns to its stable condition, electrons from the outer shells are transferred to the inner shells and in the process give off a characteristic x-ray whose energy is the difference between the two binding energies of the corresponding shells.

A SIEMENS D-500 X-ray diffraction system was used in this work for characterizing the crystals of silver in polymeric silver nanoparticle composites prepared in Chapter 5.

2.3.3 TEM, SEM and EDX

Transmission electron microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra thin specimen, interacting with the specimen as it passes through it [14-16]. An image is formed from the electrons transmitted through the specimen, magnified and focused by an
objective lens and appears on an imaging screen, a fluorescent screen in most TEM, plus a monitor, or on a layer of photographic film, or to be detected by a sensor such as a CCD camera.

The TEM is used in both material science, metallurgy and the biological sciences. In all cases the specimens must be very thin and able to withstand the high vacuum present inside the instrument.

For biological specimens, the maximum specimen thickness is roughly 1 micrometre. To withstand the instrument vacuum, biological specimens are typically held at liquid nitrogen temperatures after embedding in vitreous ice, or fixated using a negative staining material such as uranyl acetate or by plastic embedding. Typical biological applications include tomographic reconstructions of small cells or thin sections of larger cells and 3-D reconstructions of individual molecules via Single Particle Reconstruction.

In material science or metallurgy the specimens tend to be naturally resistant to vacuum, but must be prepared as a thin foil, or etched so some portion of the specimen is thin enough for the beam to penetrate. Preparation techniques to obtain an electron transparent region include ion beam milling and wedge polishing. The focused ion beam (FIB) is a relatively new technique to prepare thin samples for TEM examination from larger specimens. Because the FIB can be used to micro-machine samples very precisely, it is possible to mill very thin membranes from a specific area of a sample, such as a semiconductor or metal. Materials that have dimensions small enough to be electron transparent, such as powders or nanotubes, can be quickly produced by the deposition of a dilute sample containing the specimen onto support grids. The suspension is normally a
volatile solvent, such as ethanol, ensuring that the solvent rapidly evaporates allowing a sample that can be rapidly analysed.

In this work, silver nanoparticle polymeric composites were characterized by a TEM (JOEL TEM-100CXII). The specimen was prepared by diluting silver nanoparticle polymeric composite in ethanol and was casted on copper support grids. The results were shown in Chapter 5 and Chapter 6.

The scanning electron microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern [17,18]. The electrons interact with the atoms that make up the sample producing signals that contain information about the sample's surface topography, composition and other properties such as electrical conductivity.

All samples must also be of an appropriate size to fit in the specimen chamber and are generally mounted rigidly on a specimen holder called a specimen stub. Several models of SEM can examine any part of a 6-inch (15 cm) semiconductor wafer, and some can tilt an object of that size to 45 degrees.

For conventional imaging in the SEM, specimens must be electrically conductive, at least at the surface, and electrically grounded to prevent the accumulation of electrostatic charge at the surface. Metal objects require little special preparation for SEM except for cleaning and mounting on a specimen stub. Nonconductive specimens tend to charge when scanned by the electron beam, and especially in secondary electron imaging mode, this causes scanning faults and other image artefacts. They are therefore usually coated with an ultra thin coating of electrically-conducting material, commonly gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation. Conductive
materials in current use for specimen coating include gold, gold/palladium alloy, platinum, osmium, iridium, tungsten, chromium and graphite

Energy dispersive X-ray (EDX) spectroscopy is an analytical technique used for the elemental analysis or chemical characterization of a sample. As a type of spectroscopy, it relies on the investigation of a sample through interactions between electromagnetic radiation and matter, analyzing x-rays emitted by the matter in response to being hit with the electromagnetic radiation. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing x-rays that are characteristic of an element’s atomic structure to be identified uniquely from each other. EDX is always equipped with SEM to analyze a sample.

The morphology and composition of silver and silver-platinum alloy targets and related sputter coatings containing silver or silver-platinum alloys used in this work were characterized by a SEM (ZEISS supra 35VP) without further treatment. The results were shown in Chapter 3 and Chapter 4. The morphology and compositions of the polymeric silver nanoparticle composites prepared in this work were characterized by a SEM (JEOL JXA-8600) equipped with an EDX. The specimen was coated on a microscopy slide and sputter coated with gold to produce a conductive surface. The results were shown in Chapter 5.

2.3.4 Raman Spectroscopy

Raman spectroscopy is a technique used in condensed matter physics and chemistry to study vibrational, rotational, and other low-frequency modes in a system. It relies on inelastic scattering, or Raman scattering of monochromatic light, usually from a laser in the visible, near infrared, or near ultraviolet range.
[8,19,20]. The laser light interacts with phonons or other excitations in the system, resulting in the energy of the laser photons being shifted up or down. The shift in energy gives information about the phonon modes in the system. Infrared spectroscopy yields similar, but complementary information.

Typically, a sample is illuminated with a laser beam. Light from the illuminated spot is collected with a lens and sent through a monochromator. Wavelengths close to the laser line, due to elastic Rayleigh scattering, are filtered out while the rest of the collected light is dispersed onto a detector.

The Raman effect occurs when light impinges upon a molecule and interacts with the electron cloud of the bonds of that molecule. The incident photon excites one of the electrons into a virtual state. For the spontaneous Raman effect, the molecule will be excited from the ground state to a virtual energy state, and relax into a vibrational excited state, which generates Stokes Raman scattering. If the molecule was already in an elevated vibrational energy state, the Raman scattering is then called anti-Stokes Raman scattering (Figure 2.12).
A molecular polarizability change, or amount of deformation of the electron cloud, with respect to the vibrational coordinate is required for the molecule to exhibit the Raman effect. The amount of the polarizability change will determine the Raman scattering intensity, whereas the Raman shift is equal to the vibrational level that is involved.

Surface Enhanced Raman Spectroscopy (SERS) is used to characterize a silver or gold colloid or a substrate containing silver or gold. Surface plasmons of silver and gold are excited by the laser, resulting in an increase in the electric fields surrounding the metal. Given that Raman intensities are proportional to the electric field, there is large increase in the measured signal.

This work preliminarily studied the properties of silver nanoparticle composite by a Raman Spectroscopy (JOBINYVON) with a laser beam of 514 nm. The results were shown in Chapter 5.
2.3.5 Atomic Absorption Spectroscopy (AAS)

The atomic absorption spectroscopy uses the principle of quantum chemistry to detect the presence of certain metals and determine the concentration of those metals in samples. Quantum mechanics or quantum chemistry describes the geometry of atoms and molecules in terms of complex mathematical expressions. It also describes the relative states of atomic matter [11,12].

All atoms and their components have energy. The most stable energy level at which an atom exists is referred to as its ground state. Although the precise energy state for an atom cannot be measured, it is usually possible to measure changes to its energy relative to its ground state.

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It relies therefore heavily on Beer-Lambert law.

This work used AA technique to measure the concentration of silver leaching out from a first-aid bandage coated with silver nanoparticle polymeric composite in water. The results were shown in Chapter 6.
2.4 References:

<table>
<thead>
<tr>
<th>No.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td><a href="http://www.answers.com/topic/galvanic-cell">http://www.answers.com/topic/galvanic-cell</a></td>
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</tbody>
</table>
Chapter 3 Silver Dissolution Behaviour from Silver and Silver-Platinum Alloys
Abstract

This chapter studied the dissolution behaviour of silver from pure silver and a series of silver-platinum alloys by Cyclic Voltammetry (CV) measurement in electrolytes with the absence and in the presence of chloride. Silver dissolved to form silver ion in NaClO₄ solution and showed Tafel control process while silver chloride formed in a solution containing chloride. Addition of platinum to the Pt-Ag alloys increased the potential values for silver dissolution and the zero current potential in both chloride containing and chloride free electrolytes studied by cyclic voltammetric experiment.

The morphology of the surfaces and compositions of the samples in different conditions were characterized with Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) analysis.

The effect of platinum on the silver dissolution was investigated with Zero Resistance Amperometry (ZAR) technique. Electrical Impedance Spectroscopy (EIS) method was applied to build a model for the mechanism of silver dissolution from silver-platinum alloy. A double layer-film mechanism was proposed for silver dissolution from silver and silver-platinum alloy in sodium chloride solution.
3.1 Introduction

Medical devices coated with silver have recently proved their effectiveness in reducing infections [1-3]. Silver is active against a broad spectrum of bacteria with the antimicrobial activity being proportional to the free silver ion concentration [4]. However, in an environment containing albumin and halide ions, the antibacterial activity of silver ions will decrease as a result of specific binding with albumin and precipitation as insoluble silver halide. This can cause cytotoxicity [4-7]. Therefore, controlling the concentration of free silver ions is one of the key requirements for silver used in hygienic coatings and biomedical applications.

Numerous investigations have focussed on the formation and the reduction of AgCl films on silver electrodes and the dissolution of silver affected by pH and the concentrations of Cl$^-\$ in solution [8-15]. Burstein et al. [12,16] found that the first monolayer formed on the surface of a silver electrode in chloride electrolyte is probably a mixture of AgOH and AgCl before the second monolayer of AgCl occurs at relatively higher potentials. The potentials for both conditions are below the Ag/AgCl reversible potential. A radiotracer study of anion adsorption at a silver electrode in acidic media was carried out by Horanyi et al [17] and it concluded that the onset of anodic dissolution of silver is preceeded by significant adsorption of the anion. Formation of AgCl on the electrode surface was studied by Roy et al. [18] who used Surface Enhanced Raman Scattering to confirm the adsorption of Cl$^-\$ on a Ag electrode. X-ray photoelectron spectroscopy (XPS) and ion scattering spectroscopy (ISS) were employed to investigate the electrochemical double layer on silver electrodes in chloride solution by Hecht.
and Strehblow [19,20]. Their results showed that approximately one monolayer of Cl\(^-\) was adsorbed prior to the electrochemical formation of bulk AgCl.

The kinetics of chloride ion adsorption and the mechanism of AgCl layer formation on the (111), (100) and (110) faces of silver were investigated by Jovic et al. [9]. Katan et al. [13] and Jin et al. [8] studied the electrochemical formation and reduction of thick porous AgCl layers on a silver substrate. Katan suggested that the mechanism of the formation and reduction of porous AgCl layers involved the diffusion of ions in solution and the surface diffusion of metallic silver atoms to the growing sites on the silver surface. Jin et al. suggested that during the anodic growth of AgCl, partially solvated Ag\(^+\) ions in inter-grain micro-channels were the main charge carriers.

Brolo et al. [21] investigated the electrochemical oxidation of silver in both chloride containing and chloride free solutions by probe beam deflection (PBD) and cyclic voltammetry (CV). It was confirmed that the Ag\(^+\) ions were the main product of the silver oxidation in the absence of the halide while the formation of a AgCl(s) film was observed for the oxidation of silver in the solutions containing Cl\(^-\) and the degree of formation of Ag\(^+\) ions depended on the porosity of AgCl film.

The kinetics of anodic dissolution of silver and Ag-Au alloys (0.1-30 wt.% Au) in aqueous alkaline solution under the conditions of the formation of silver oxides has been examined by Vvedenskii et al. [22]. It was established that the anodic formation and cathodic reduction of Ag\(_2\)O on silver and alloys are controlled by migration of silver ions via the oxide layer.
This work focuses on the dissolution behaviour of silver from silver-platinum alloys under the conditions suitable for applications in medical devices and in hygienic coatings.

3.2 Experimental

3.2.1 Cyclic Voltammetric (CV) set-up

Cyclic Voltammetry (CV) experiment was carried out in a flat cell connected to a Solartron 1287 potentiostat controlled with Corrware software. The Setup conditions are shown in Table 3.1 and Table 3.2.

Table 3.1 Setup for Solartron 1287

<table>
<thead>
<tr>
<th>Current range</th>
<th>Auto</th>
<th>IR compensation</th>
<th>Off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current limitation</td>
<td>Auto</td>
<td>Low Pass Filter</td>
<td>Off</td>
</tr>
<tr>
<td>Measurement</td>
<td>$E, I$</td>
<td>Band Width</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pstat</td>
<td>$C$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gstat</td>
<td>$C$</td>
</tr>
<tr>
<td>Sweep Type</td>
<td>stepped</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.2 Setup for Cell

<table>
<thead>
<tr>
<th>Electrode</th>
<th>Surface area: 0.785 cm$^2$</th>
<th>WE: Pt-Ag targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density:</td>
<td></td>
<td>CE: Platinum</td>
</tr>
<tr>
<td>Equivalent weight:</td>
<td></td>
<td>RE: SCE (Saturated Calomel Electrode)</td>
</tr>
<tr>
<td>Stern-Geary Coef (mv):</td>
<td>26</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>0.89%wt NaCl solution (saline solution)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Corrosion Units:</th>
<th>mpy</th>
</tr>
</thead>
</table>

A cylindrical shaped electrochemical flat cell (EG&G PARC, KO235) was utilized with a normal three-electrode configuration in this work. In this system a Teflon ring seal defined the geometric working electrode area at 0.785 cm$^2$.

$\text{NaClO}_4$ (SIGMA-ALDRICH), $\text{NaCl}$ (SIGMA-ALDRICH), Ringer’s (OXOID), and Phosphate Buffered Saline (PBS, OXOID) solutions were prepared from the reagent grade chemicals with de-ionized water. A solution of volume of 200ml was used for all measurement in this work. De-oxygenation of the solution was accomplished by passing nitrogen (BOC, 99.999%) through the solution in the cell before and maintaining a blanket of nitrogen above the solution during the experiment.

1. The electrolyte was 0.89%wt. (0.152M) NaCl solution. The samples were studied in both aerated and de-aerated solution. The de-aerated electrolyte consisted of the NaCl solution with nitrogen bubbling for 2 hour before the experiment started.
2. Phosphate buffered saline solution (OXOID) and Ringer’s solution-1/4 strength ringer solution (OXOID) were also employed for examining the CV behavior of these targets. The compositions of the solution are listed below in Table 3.3.

3. 0.152M NaClO$_4$ and 0.152M KNO$_3$ solution were prepared for measuring the behavior of silver and silver-platinum alloys by CV without Cl$^-$ in the solution.

Table 3.3 Compositions of Phosphate Buffered Saline solution and Ringer’s solution

<table>
<thead>
<tr>
<th></th>
<th>Phosphate buffered saline solution</th>
<th>Ringer’s solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>8.0</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>0.2</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>Disodium hydrogen phosphate</td>
<td>1.15</td>
<td>Calcium chloride (6H$_2$O)</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>0.2</td>
<td>Sodium bicarbonate</td>
</tr>
<tr>
<td>pH=7.3</td>
<td></td>
<td>pH=7.0</td>
</tr>
</tbody>
</table>

The reference electrode used throughout the investigation was a Saturated Calomel Electrode (SCE, 242.4 mV vs. NHE). The counter electrode was a platinum mesh with a surface area of 8 cm$^2$. Ag and Ag-Pt (0.5-5% wt. Pt) alloys (manufactured for magnetron target by Testbourne Ltd.) discs, generally used as targets for sputtering, with 5mm thickness and 65mm diameter were clamped at the end of the flat cell and served as working electrodes. The working electrode
was washed in acetone in an ultrasonic bath for 15 minutes to remove any organic impurity on the surfaces of the sample prior to use. The electrodes were pretreated by polishing with 1200 silicon carbide grinding paper and rinsed with de-ionized water to produce a fresh surface. These samples were dried in an environment with a positive pressure of nitrogen.

3.2.2 Zero Resistance Amperometry (ZRA) measurement

Measurements of galvanic currents between silver and platinum coupled metals are based on the use of zero resistance amperometry (ZRA). A Solartron 1287 potentiostat controlled with Corrware software were setup as a ZRA. The working electrode (WE) wire and the reference electrode 2 (RE2) wire combined to one served as working electrode 1 (WE1). The counter electrode (CE) wire was not used. The ground wire connected to pure platinum served as working electrode 2 (WE2). WE1 was the silver or silver platinum alloy targets described in section 3.2.1. The surface area of 0.785 cm$^2$ of the targets was in contact with the electrolyte. WE2 was a platinum foil with an area of 8 cm$^2$ or a mini platinum electrode, which was prepared with a series of platinum wires in 1mm diameter cast in resin. The reference electrode was Saturated Calomel Electrode (SCE). Both WE1 and WE2 were polished with 1200 silicon carbide grinding paper and washed in acetone and de-ionized water in an ultrasonic bath for 15 minutes prior to use.
3.2.3 **Electrochemical Impedance Spectroscopy (EIS) experiment**

EIS experiment was carried out in a three-electrode cylindrical shaped electrochemical flat cell (EG&G PARC, KO235) connected to a Solartron 1287 potentiostat controlled with ZPlot-ZView software. The working electrode was silver and silver-platinum alloy targets polished with 1200 silicon carbide grinding paper and washed de-ionized water in an ultrasonic bath for 15 minutes prior to use. The reference electrode was a saturated calomel electrode (SCE) and the counter electrode was a platinum foil with an area of $8 \text{ cm}^2$. The electrolyte was 0.89%wt. NaCl solution exposed to air without de-oxygen treatment. A 10mV peak-to-peak signal and a frequency range from $10^2 \text{ Hz}$ to $10^4 \text{ Hz}$ were applied.

3.2.4 **SEM**

The morphology of surface of each sample was characterised with Scanning Electron Microscopy (SEM, ZEISS Supra 35 VP) at different potentials in the CV and the composition of the surfaces were analyzed by EDX.
3.3 Results and Discussions

3.3.1 The dissolution behavior of silver from Ag and Ag-Pt alloy samples measured by CV in 0.152M NaClO₄ solution

![Cyclic Voltammogram of Ag and Ag-Pt alloy samples in 0.152M NaClO₄ solution; scan rate: 10mV/s; the sweeping range: -0.3 to +0.8 V vs SCE](image)

The behaviour of silver dissolution from pure silver and from silver-platinum alloys (0.5-5%Pt-Ag) in 0.152M sodium perchlorate solution was investigated by cyclic voltammetry (CV). The potential sweeping range was from -0.3 V to +0.8 V vs. SCE. The potential scan rate was 10mV/s. Figure 3.1 showed the process of silver dissolution from samples of silver and silver-platinum alloys which displayed similar behaviour. Silver started to dissolve to form Ag⁺ at around
+0.31V vs. SCE from pure silver and at around +0.36V vs. SCE for all the silver-platinum alloy samples and the current increase rapidly and then became linear which indicated that silver dissolution was initially under activation control followed by diffusion control to resistive control process [22]. With increasing amount of platinum in the alloys, the anodic current dropped. The more platinum contained in the samples, the slower the silver dissolution rate. The ability of the silver to be oxidized at the surface is influenced by the level of Pt in the alloy.

At the beginning of the forward sweeping process (Figure 3.1a), there was a cathodic and anodic process in the potential range of -0.3V to +0.2V vs. SCE on both pure silver and silver-platinum alloy samples. This was caused by the reduction of oxygen on the surface of silver and silver-platinum samples according to the reactions shown below [23-25]:

\[
2\text{H}_2\text{O} + \text{O}_2 + 4\text{e}^- = 4\text{OH}^- \quad \text{E}^o = 0.16 \text{ V vs. SCE} \quad (3-1)
\]

\[
2\text{Ag} + 2\text{OH}^- = \text{Ag}_2\text{O} + \text{H}_2\text{O} + 2\text{e}^- \quad \text{E}^o = 0.10\text{V vs. SCE} \quad (3-2)
\]

The potential for the reduction of oxygen on the silver-platinum alloys was lower than that on pure silver. This indicates that platinum facilitated the reduction of oxygen compared to pure silver. With continuously increasing the sweeping potential to +0.3V vs. SCE, the process went to a stage of passivation or reorganized surfaces of Ag$_2$O on the samples by reaction of silver and the OH$^-$ that had been produced by the reduction of oxygen in the solution [22].
When silver started to oxidize to silver ions, the dependence of current on potential was exponential in the potential range of +0.36 to +0.4 V vs. SCE. It suggested that at this stage the dissolution was in an activation control process.

Figure 3.1a The transformed forward sweep of Cyclic Voltammogram in Figure 3.1; 0%Pt-Ag and 5%Pt-Ag samples in 0.152M NaClO₄ solution; scan rate: 10mV/s
The Tafel equations (shown below) were worked out for pure silver sample and silver-platinum alloys:

Tafel equation in the case of a cathodic current is \( \ln i = \ln i_0 - \frac{nF}{RT}(E - E^\theta) \) and for an anodic current is \( \ln i = \ln i_0 + (1-\alpha)nF/RT(E - E^\theta) \), which is simplification of equation (2-18).

Over the pure silver sample, the Tafel equation was:

\[
\log_{10} i_{Ag} = 10.602E-7.752
\]

\[
E_{Ag} = 0.094\log_{10} i_{Ag} + 0.731
\]

\[
\alpha_{Ag} = 0.38
\]

Over the silver-platinum samples, the Tafel equation shown as:

\textbf{Figure 3.1b} The transformed backward sweep of Cyclic Voltammogram in Figure 3.1; 0\%Pt-Ag and 5\%Pt-Ag samples in 0.152M NaClO\(_4\) solution; scan rate: 10mV/s
\[
\log_{i_{Ag-Pt}} = 7.745 \times 10^{-6.460}
\]
\[
E_{Ag-Pt} = 0.129 \log_{i_{Ag-Pt}} + 0.834
\]
\[
\alpha_{Ag-Pt} = 0.54
\]

With a further increase of the sweeping potential, the dissolution became limited by diffusion process. This could result from the movement of Ag atoms in the samples and/or the desorption of \(Ag^+\) from the surfaces diffusing into the solution. With increasing the amount of platinum in the alloys, the current dropped. The decrease in current is due to a combination of smaller active surface area along with platinum interfering with the migration of silver atoms on the surface.

For the backward swept process from \(+0.8V\) to \(-0.3V\) vs. SCE (Figure 3.1b), silver oxidized to silver ions over both pure silver and silver-platinum samples from \(+0.8V\) vs. SCE. With further decreasing the potential, silver ions started to reduce to silver atoms. The potential for silver ion reduction at silver-platinum samples was higher than that of pure silver. The potential where the silver ion reduction current dropped to zero was defined at zero current potential. The more platinum contained in the alloys, the higher value of zero current potential for silver ion reduction.

The reduction current was much smaller than the oxidation current and also was evident in Figure 3.1. Less than 5%wt. dissolved silver was reduced back and most dissolved silver diffused into solution.
3.3.2 Silver dissolution from pure silver and silver-platinum alloys in Ringer’s solution

With 0.28wt.% Cl\(^-\) in Ringer’s solution, the dissolution behaviour of silver from silver and silver-platinum alloys were tested from -0.3 V to +0.8V vs. SCE for three continuous cycles where the scan rate was 10mV/s. All the samples had a similar behaviour on the first cycle. Figure 3.2 – Figure 3.4 showed the results of pure silver and 5%wt Platinum silver alloy as examples. The dissolution behaviour of silver was different from those measured by CV in chloride free solutions (Figure 3.1).

Figure 3.2 First cycle of CV for Ag and Ag-Pt (5%wt. Platinum) alloy samples in Ringer’s solution containing 0.28%wt. Cl\(^-\); scan rate: 10 mV/s
Chapter 3 Silver Dissolution Behaviour from Silver and Silver-Platinum Alloys

Figure 3.2a Transformed voltammogram of CV in Figure 3.2; Ag and Ag-Pt (5%wt. Platinum) alloy samples in Ringer's solution containing 0.28%wt. Cl⁻; scan rate: 10mV/s

Figure 3.3 Second cycle of CV measurement for Ag and Ag-Pt (5%wt. Platinum) alloy samples in Ringer’s solution containing 0.28%wt. Cl⁻; scan rate: 10mV/s
Figure 3.3a Transformed voltammogram of CV in Figure 3.3; Ag and Ag-Pt (5%wt. Pt) alloy samples in Ringer’s solution containing 0.28%wt. Cl⁻; scan rate: 10mV/s

Figure 3.4 Third cycle of CV measurement for Ag and Ag-Pt (5%wt. Platinum) alloy samples in Ringer’s solution containing 0.28%wt. Cl⁻; scan rate: 10mV/s
The potential for silver dissolution in Ringer’s solution started from around 0.08 V vs. SCE (Figure 3.2). With increasing the amount of platinum, this potential did slightly shift to higher values: 0.077 V for pure silver, 0.078 V for 0.5wt.%Pt-Ag, 0.083 V for 1.5wt.%Pt-Ag, 0.086 V for 3wt.%Pt-Ag, and 0.095 V for 5wt.%Pt-Ag sample, respectively. It started with the formation of silver chloride on the surface of the samples in a diffusion control process. Before the silver dissolution took place, reduction of oxygen proceeded at a potential -0.1 V vs SCE. For the backward sweeping process, the zero current potential was 0.076 V vs. SCE on pure silver sample (Figure 3.2); for 5%Pt-Ag silver, the zero current potential was 0.145V vs. SCE. The zero current potential for silver platinum alloys was higher

Figure 3.4a Transformed voltammogram of CV in Figure 3.4; Ag and Ag-Pt (5%wt. Platinum) alloy samples in Ringer’s solution containing 0.28%wt. Cl\(^{-}\); scan rate: 10mV/s
than that for pure silver. The difference in zero current potential between silver and the alloy was greater in Cl\(^-\) containing solutions than in Cl\(^-\) free solution.

For the second and third cycles (Figure 3.3 and Figure 3.4), at the beginning of the scan for each cycle, the residual silver chloride from previous scan cycle was reduced. When the sweeping potential was high enough, silver started dissolving to form silver chloride. Pt-Ag alloy samples showed a dissolution peak on the forward scan (Figure 3.3), followed by a straight line. The dissolution peaks indicated a maximum rate for the formation of a silver chloride layer on the surfaces of the samples. The straight line was caused by the dissolution of silver to silver ions through silver chloride layer [26]. This suggested that the amount of platinum affected the formation of silver chloride on the surface of the samples, which dominated the dissolution behaviour of silver from silver-platinum alloys.

The dissolution of silver in Ringer’s solution was also investigated by CV from -0.9 V to +0.8 V vs. SCE (Figure 3.5 – Figure 3.7). This was to measure the dissolution of silver under the conditions with complete reduction of silver chloride to silver.

The dissolution behaviour of silver from silver and silver-platinum alloys was similar for the first cycle. The silver chloride was completely reduced at -0.9 V vs. SCE. After determining the amount of silver oxidized to silver chloride and the amount of silver chloride completely reduced back to silver, these two values were similar. This indicated that the formation of silver chloride was grown on the top of the surface and completely reduced back to silver that was still sitting on the top of the surface.
Chapter 3 Silver Dissolution Behaviour from Silver and Silver-Platinum Alloys

**Figure 3.5** First cycle of CV measurement for Ag and Ag-Pt (5%wt. Platinum) alloy samples in Ringer’s solution contained 0.28%wt. Cl⁻; scan rate: 10mV/s

**Figure 3.5a** Transformed voltammogram of CV in Figure 3.5; Ag and Ag-Pt (5%wt. Platinum) alloy samples in Ringer’s solution containing 0.28%wt. Cl⁻; scan rate: 10mV/s
Figure 3.6 Second cycle of CV measurement for Ag and Ag-Pt (5%wt. Platinum) alloy samples in Ringer’s solution contained 0.28%wt. Cl⁻; scan rate: 10mV/s

Figure 3.6a Transformed voltammogram of CV in Figure 3.6; Ag and Ag-Pt (5%wt. Platinum) alloy samples in Ringer’s solution containing 0.28%wt. Cl⁻; scan rate: 10 mV/s
Figure 3.7 Third cycle of CV measurement for Ag and Ag-Pt (5% wt. Platinum) alloy samples in Ringer’s solution contained 0.28% wt. Cl⁻; scan rate: 10mV/s

Figure 3.7a Transformed voltammogram of CV in Figure 3.7; Ag and Ag-Pt (5% wt. Platinum) alloy samples in Ringer’s solution containing 0.28% wt. Cl⁻; scan rate: 10mV/s
In Figure 3.5 -3.7, it can be seen that there is less Faradaic current for the 5%wt. Pt alloy compared to pure silver. This is seen as the exhaustive reduction of AgCl at an earlier potential on the reverse sweep. Since this decrease is more than 5% of the charge, it can be seen that this effect is not merely due to a 5% decrease in active area but that the platinum has an influence on dissolution and nucleation processes at the electrode surface as mentioned previously.

3.3.3 Silver dissolution from pure silver and silver-platinum alloys in Phosphate Buffered Saline (PBS) solution
The dissolution behaviour of silver from silver and silver-platinum alloys was studied in Phosphate Buffered Saline (PBS) solution by CV from -0.3 to +0.8 V vs. SCE and the scan rate was 10 mV/s. The composition of PBS solution was listed in Table 3.3. The concentration of Cl\(^-\) was 0.82wt.% (0.28wt.% in Ringer’s solution) and with phosphate introducing in PBS solution. The results are shown in Figure 3.8 – Figure 3.9. In general, the dissolution of silver from pure silver and silver-platinum alloys in PBS solution had similar behaviour, but it was different from those in Ringer’s solution.
Figure 3.8 Cyclic Voltammogram of Ag in PBS solution for the first three cycles; scan rate: 10mV/s

Figure 3.8a Transformed voltammogram of CV in Figure 3.8; Ag in PBS solution for the first three cycles; scan rate: 10mV/s
Figure 3.9 Cyclic Voltammogram of Ag-Pt alloy (3%wt. Platinum) in PBS solution for the first three cycles; scan rate: 10mV/s

Figure 3.9a Transformed voltammogram of CV in Figure 3.9; Ag PBS solution for the first three cycles; scan rate: 10mV/s
Silver started dissolving from 0.057 V vs. SCE for the pure silver sample in PBS solution and from 0.058 to 0.064 V vs. SCE for the silver-platinum alloy samples. The dissolution currents were about 0.016 Amps cm\(^{-2}\) at +0.8V for the first cycle and were higher than those in Ringer’s solution (about 0.006 Amps/cm\(^2\)). For the second and third cycles, the rate for the formation of silver chloride reached a maximum value and silver dissolved into silver ions through the silver chloride film. In the third cycle, less silver chloride formed and more silver ions formed compared to the second cycle.

3.3.4 Silver dissolution from silver and silver-platinum alloys in 0.89%wt. NaCl saline solution

Silver dissolution behaviour from silver and silver-platinum alloys was investigated in the potential ranges of -0.3V to +0.8V and -0.9V to +0.8V in aerated and de-aerated 0.89%wt NaCl solutions. This solution is typically used as a sterile saline solution in healthcare.

Figure 3.10-3.10a showed the CV results of silver and silver-platinum alloys in air-exposed 0.89%wt NaCl solution. The scan rate was 10mV/s and the swept potential ranged from -0.3V to +0.8V. In the first cycle silver was oxidized in the forward process and the AgCl layer formed on the surface. When the potential swept back from +0.8V to -0.3V, AgCl layer was partially reduced. The backward curves showed two current peaks. This may indicate that two oxidation processes were probably occurred at the same time according to the reactions:

\[
\begin{align*}
Ag + Cl^- &= AgCl + e^- & E^0 &= -0.019 \text{ V vs. SCE} \quad (3-3) \\
Ag &= Ag^+ + e^- & E^0 &= 0.558 \text{ V vs. SCE} \quad (3-4)
\end{align*}
\]
Chapter 3 Silver Dissolution Behaviour from Silver and Silver-Platinum Alloys

Figure 3.10 Cyclic Voltammogram of Ag in 0.89%wt. NaCl (0.152M) solution for the first three cycles; scan rate: 10mV/s

Figure 3.10a Transformed voltammogram of CV in Figure 3.10; Ag in 0.89%wt. NaCl solution for the first three cycles; scan rate: 10mV/s
Chapter 3 Silver Dissolution Behaviour from Silver and Silver-Platinum Alloys

Figure 3.11 Cyclic Voltammogram of Ag in 0.89%wt. NaCl (0.152M) solution for the first three cycles; scan rate: 10mV/s

Figure 3.11a Transformed voltammogram of CV in Figure 3.11; Ag in 0.89%wt. NaCl (0.152M) solution for the first three cycles; scan rate: 10mV/s
The peak occurring on the backward sweeping process showed silver oxidized to silver ions in the case where there is a lack of Cl ions within the film and diffused through the silver chloride film. Alternatively the peak on the reverse sweep may arise from nucleation sites that facilitate a rapid growth of silver chloride nuclei. For the second cycle, in the forward process, there was a peak current at the potential of ca. 0.4V and when the potential increased over 0.5V, the current increased linearly. The straight increase in current at the potential over 0.5V was caused by the dissolution of silver to silver ions diffusing through the porous AgCl layer which is characterized with SEM and will be shown in the following section.

In the third cycle, the peak current occurred in the forward process was shifted to lower potential than those in the second cycles. This may suggest that more AgCl film accumulated on the surfaces from the previous two cycles and the dissolution of silver to silver ions occurred at a lower potential as well.

Figure 3.11-3.11a showed the CV results of silver investigated in the potential range of -0.9V to +0.8V vs. SCE in 0.89%wt. NaCl solution. Silver dissolution had the similar behavior compared to that in the range of –0.3V to +0.8V vs. SCE. However, the swept potential range had a significant effect on the CV behavior for the second and third cycles. In the forward process, the silver was oxidized and the AgCl layer formed while in the backward process, the layer of AgCl on the surfaces was completely reduced to silver. The reduced silver facilitated the subsequent dissolution of silver and the formation of AgCl film on the surfaces of all samples. The dissolution of silver to silver ions was observed at the sweeping potential of +0.8V vs SCE. The reduction charge was still similar to total
oxidation charge of silver. This also indicated that silver chloride was confined to
the surface.

**Figure 3.12** Cyclic Voltammogram of Ag-Pt alloy (3%wt. Platinum) in
0.89%wt. NaCl (0.152M) solution for the first three cycles; scan rate: 10mV/s

**Figure 3.12a** Transformed voltammogram of CV in Figure 3.12; Ag in
0.89%wt. NaCl (0.152M) solution for the first three cycles; scan rate: 10mV/s
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Figure 3.13 Cyclic Voltammogram of Ag-Pt alloy (3% wt. Platinum) in 0.89% wt. NaCl (0.152M) solution for the first three cycles; scan rate: 10mV/s

Figure 3.13a Transformed voltammogram of CV in Figure 3.13; Ag in 0.89% wt. NaCl (0.152M) solution for the first three cycles; scan rate: 10mV/s
For all samples of silver and silver-platinum alloys, the CV behaviour was similar. Figure 3.12 and Figure 3.13 show an example of silver-platinum alloys (3%wt. Platinum alloy) studied by CV in 0.89%wt.NaCl solution. The difference between them and the CV results of pure silver was that the current density decreased with an increase of the levels of platinum in the samples.

Figure 3.14 shows the effect of platinum on the current in CV measurement in aerated and de-aerated electrolyte swept from -0.9V to +0.8V. Both current and charge decreased with increasing the levels of platinum in the samples.

There was no obvious effect of oxygen in the electrolyte on the CV for all samples. Figure 3.15 shows the effect of platinum contained in the samples on the corrosion potential (Ecorr) in the sweeping ranges of -0.3V to +0.8V and -0.9V to +0.8V in both aerated and de-aerated electrolytes. In the first cycles, the corrosion potential increased with increase of the levels of platinum for all samples under all conditions examined in this work. For the second and third cycles, platinum did not have an effect on the potential where silver started to dissolve.

The zero current potential was measured at the potential where the net current was zero (Figure 3.16). In the first cycle, the silver dissolution potential increased with increase of the levels of platinum for all samples under all conditions examined in this work. For the second and third cycles, platinum did not have the effect on the potential for silver starting to dissolve because the surface was covered by the silver reduced from silver chloride in previous cycle.
Chapter 3 Silver Dissolution Behaviour from Silver and Silver-Platinum Alloys

Figure 3.14 The effect of Pt on the dissolution of silver in CV experiment in 0.89%wt NaCl solution swept from -0.9 to +0.8V
Figure 3.15 The effect of Pt on the potential where silver starts dissolving from silver and Ag-Pt alloys in 0.89%wt NaCl solution studies by CV; sweeping ranges of -0.3 to +0.8 V vs. SCE and -0.9 to +0.8 V vs. SCE; scan rate 10mV/s
On the first sweep, the silver dissolved from an alloy while on subsequent sweeps the silver dissolved from pure silver as a consequence of the previous reduction of AgCl with the Pt as an underlayer. The lack of the effect of oxygen indicates that reaction (3.1) and (3.2) previously mentioned play a small role in the dissolution of silver in chloride media.

**Figure 3.16** The effect of Pt on the potential of zero current for silver and Ag-Pt alloys in NaClO₄, Ringer’s, PBS, and NaCl solutions studies by CV; sweep ranges of -0.3 to +0.8 V vs. SCE; scan rate 10mV/s
3.3.5 The morphology and composition of Ag and Ag-Pt samples at different stages in CV studied by SEM

The morphology of the surfaces of all samples studied by CV experiment at different stages in 0.89%wt. saline solution was characterized by SEM and the compositions were measured by EDAX. Figure 3.17 and Figure 3.18 show those images taken from the sample of silver and the sample of 3%wt. platinum alloys at stages of the full oxidation of silver to silver chloride layer (top), the partial reduction of silver chloride to silver (middle) and the complete reduction of silver chloride to silver in 0.89%wt. NaCl saline solution (bottom). With silver dissolving and silver chloride forming on the surface, silver chloride preferred nucleating and formed micrometer sized particles adhering on the surface. With partial reduction of silver chloride to silver, the reduced silver nucleated and formed round areas in the surface. The completely reduced silver was in the porous state on the top of the surface.

Comparing images in Figure 3.17 and Figure 3.18, it was found that there was less spots formed on the samples containing platinum than those on pure silver samples. Therefore, the addition of platinum to silver inhibited the formation of nucleation of silver reducing from silver chloride.
Figure 3.17 The morphology of the surfaces of silver sample in 0.89%wt NaCl solution studies by CV; scan rate 10mV/s; top: silver oxidized to silver chloride at $E = +0.8V$ vs. SCE; middle: partial reduction of silver chloride at $E = -0.3V$ vs. SCE; bottom: complete reduction of silver chloride to silver at $E = -0.9V$ vs. SCE.
Figure 3.18 The morphology of the surfaces of Ag-Pt alloy (3%wt. Platinum) sample in 0.89%wt NaCl solution studies by CV; scan rate 10mV/s; top: silver oxidized to silver chloride at \( E = +0.8 \text{V} \) vs. SCE; middle: partial reduction of silver chloride at \( E = -0.3 \text{V} \) vs. SCE; bottom: complete reduction of silver chloride to silver at \( E = -0.9 \text{V} \) vs. SCE.
Figure 3.19 The surface compositions of Ag-Pt alloy (3%wt. Platinum) sample characterized by EDX: top, bare surface; bottom, silver oxidized to silver chloride at $E = +0.8V$ vs. SCE in 0.89%wt NaCl solution studies by CV; scan rate 10mV/s;
Table 3.4 Compositions of the samples and of the partial reduction surfaces studied by CV from -0.3 to +0.8V vs. SCE in 0.89wt.% NaCl solution characterized by EDX

<table>
<thead>
<tr>
<th>Samples</th>
<th>Aerated</th>
<th>De-aerated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ag wt.%</td>
<td>Cl wt.%</td>
</tr>
<tr>
<td>0Pt-Ag</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target</td>
<td>99.87</td>
<td>0.13</td>
</tr>
<tr>
<td>General</td>
<td>85.06</td>
<td>14.94</td>
</tr>
<tr>
<td>Spots</td>
<td>96.79</td>
<td>3.21</td>
</tr>
<tr>
<td>0.5Pt-Ag</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target</td>
<td>98.99</td>
<td>0.15</td>
</tr>
<tr>
<td>General</td>
<td>86.29</td>
<td>13.71</td>
</tr>
<tr>
<td>Spots</td>
<td>95.02</td>
<td>4.98</td>
</tr>
<tr>
<td>1.5Pt-Ag</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>General</td>
<td>82.24</td>
<td>17.76</td>
</tr>
<tr>
<td>Spots</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3Pt-Ag</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Target</td>
<td>94.44</td>
<td>0.11</td>
</tr>
<tr>
<td>General</td>
<td>83.09</td>
<td>16.71</td>
</tr>
<tr>
<td>Spots</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3.5 The compositions of the surfaces of the samples studied by CV from -0.9 to +0.8V vs. SCE in 0.89wt.% NaCl solution characterized by EDX

<table>
<thead>
<tr>
<th>Samples</th>
<th>Aerated</th>
<th>De-aerated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ag wt.%</td>
<td>Cl wt.%</td>
</tr>
<tr>
<td>0Pt-Ag</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecorr</td>
<td>99.92</td>
<td>0.08</td>
</tr>
<tr>
<td>complete oxidation</td>
<td>82.01</td>
<td>17.99</td>
</tr>
<tr>
<td>complete reduction</td>
<td>99.56</td>
<td>0.44</td>
</tr>
<tr>
<td>0.5Pt-Ag</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecorr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>complete oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>complete reduction</td>
<td>99.64</td>
<td>-</td>
</tr>
<tr>
<td>1.5Pt-Ag</td>
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<td></td>
</tr>
<tr>
<td>Ecorr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>complete oxidation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>complete reduction</td>
<td>99.66</td>
<td>0.34</td>
</tr>
<tr>
<td>3Pt-Ag</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecorr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>complete oxidation</td>
<td>83.61</td>
<td>15.87</td>
</tr>
<tr>
<td>complete reduction</td>
<td>99.68</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Table 3.6 Summary of silver dissolution from silver and silver-platinum alloys studied by CV from -0.3 to +0.8V vs. SCE in different electrolytes

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Samples</th>
<th>$E_{\text{corr.}}$ (Volt vs. SCE)</th>
<th>$I_{\text{max}}$ at +0.8V (Amps/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.152M NaClO₄ solution</td>
<td>0Pt-Ag</td>
<td>0.31</td>
<td>0.0117</td>
</tr>
<tr>
<td></td>
<td>0.5Pt-Ag</td>
<td>0.34</td>
<td>0.0104</td>
</tr>
<tr>
<td></td>
<td>1.5Pt-Ag</td>
<td>0.34</td>
<td>0.0098</td>
</tr>
<tr>
<td></td>
<td>3.0Pt-Ag</td>
<td>0.34</td>
<td>0.0090</td>
</tr>
<tr>
<td></td>
<td>5.0Pt-Ag</td>
<td>0.34</td>
<td>0.0090</td>
</tr>
<tr>
<td>Ringer’s solution</td>
<td>0Pt-Ag</td>
<td>0.077</td>
<td>0.00615</td>
</tr>
<tr>
<td></td>
<td>0.5Pt-Ag</td>
<td>0.078</td>
<td>0.00613</td>
</tr>
<tr>
<td></td>
<td>1.5Pt-Ag</td>
<td>0.083</td>
<td>0.00612</td>
</tr>
<tr>
<td></td>
<td>3.0Pt-Ag</td>
<td>0.086</td>
<td>0.00612</td>
</tr>
<tr>
<td></td>
<td>5.0Pt-Ag</td>
<td>0.095</td>
<td>0.00574</td>
</tr>
<tr>
<td>PBS solution</td>
<td>0Pt-Ag</td>
<td>0.057</td>
<td>0.0162</td>
</tr>
<tr>
<td></td>
<td>0.5Pt-Ag</td>
<td>0.058</td>
<td>0.0156</td>
</tr>
<tr>
<td></td>
<td>1.5Pt-Ag</td>
<td>0.058</td>
<td>0.0167</td>
</tr>
<tr>
<td></td>
<td>3.0Pt-Ag</td>
<td>0.064</td>
<td>0.0165</td>
</tr>
<tr>
<td></td>
<td>5.0Pt-Ag</td>
<td>0.064</td>
<td>0.0165</td>
</tr>
<tr>
<td>0.89wt.% NaCl (0.152M)</td>
<td>0Pt-Ag</td>
<td>0.054</td>
<td>0.0115</td>
</tr>
<tr>
<td>solution</td>
<td>0.5Pt-Ag</td>
<td>0.060</td>
<td>0.0105</td>
</tr>
<tr>
<td></td>
<td>1.5Pt-Ag</td>
<td>0.067</td>
<td>0.0106</td>
</tr>
<tr>
<td></td>
<td>3.0Pt-Ag</td>
<td>0.077</td>
<td>0.0109</td>
</tr>
<tr>
<td></td>
<td>5.0Pt-Ag</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The compositions of the surfaces of the samples at different stages in the CV were characterized by EDX (Figure 3.19). Table 3.4 and Table 3.5 show the results. The levels of platinum observed on the surfaces of the samples of silver-platinum
alloys following CV sweeps were lower than that in the bulk of the samples. This confirms that the silver layers were formed on top of the underlying platinum.

Table 3.6 summarized silver dissolution behaviour from pure silver and silver-platinum alloys in both chloride containing and chloride free solutions studied by CV from -0.3 to +0.8 V vs. SCE.

### 3.3.6 The effect of Platinum on silver dissolution behavior studied by CV

![Model for the mixed potential of silver and silver-platinum alloy. The slopes are in the relative agreement with the measured polarization curve slope.](image-url)

**Figure 3.20** Model for the mixed potential of silver and silver-platinum alloy. The slopes are in the relative agreement with the measured polarization curve slope.
An increased percentage of platinum in silver-platinum alloys resulted in the increase in silver dissolution potential in both chloride containing solutions and chloride free solutions (Figure 3.20). The more platinum present in the samples, the higher the value of the silver dissolution potential and so a higher potential should be applied to ensure silver dissolves.

In the presence of chloride, a silver chloride layer is formed during the dissolution process. Platinum affected the morphology of silver chloride layer formed on the surfaces of each samples.

3.3.7 The effect of Platinum on silver dissolution behavior studied by Zero Resistance Amperometry (ZRA)

To further investigate the effect of platinum on the effect of silver dissolution from silver-platinum alloys in 0.89wt.% sodium chloride solution, Galvanic measurement was setup as two electrodes were connected in the electrolyte and the current and potential (vs. SCE) were measured by the Solartron 1287 potentiostat controlled with Corrware software. The results are shown in Figure 3.21 and Figure 3.23.

In Figure 3.21, the cathode was a piece of platinum foil with a physical surface area of 8 cm². The anode was the silver-platinum alloys clamped in one side of the flat cell and the area was 0.785 cm². The measurement time was over 20 hours. It was found that the potential for silver dissolution from pure silver was the lowest in these samples while the current was the highest for pure silver sample. With increasing the amount of platinum in the alloys, the potential for silver dissolution increased but the current dropped for the galvanic reaction taking place between
pure platinum and silver-platinum alloys. Figure 3.22 shows the model, predicting an increasing in potential with platinum loading. The open circuit potential automatically measure by the solartron increased with increasing in the amount of platinum containing in the Ag-Pt alloys (Figure 3.25).

**Figure 3.21** Galvanic study of silver dissolution between platinum and silver-platinum alloys in 0.89wt% NaCl solution; 0PtAg, pure silver; 0.5PtAg, 0.5wt%Pt-Ag alloy; 1.5PtAg, 1.5wt%Pt-Ag alloy; 3PtAg, 3wt%Pt-Ag alloy; 5PtAg, 5wt%Pt-Ag alloy;
Another experiment was setup, the anode was fixed as pure silver (surface area: $0.875 \, \text{cm}^2$), and the cathode was pure platinum with changing the surface areas from $0.00875 \, \text{cm}^2$ (1mPt-Ag), $0.0175 \, \text{cm}^2$ (2mPt-Ag) and $0.0425 \, \text{cm}^2$ (5mPt-Ag).

**Figure 3.22** Model of the mixed potential of silver dissolution between platinum and silver-platinum alloys studied zero resistance ammeter in 0.89wt% NaCl solution; 0PtAg, pure silver; 0.5PtAg, 0.5wt%Pt-Ag alloy; 1.5PtAg, 1.5wt%Pt-Ag alloy; 3PtAg, 3wt%Pt-Ag alloy; 5PtAg, 5wt%Pt-Ag alloy;
The results are shown in Figure 3.23. With increasing the amount of platinum, the dissolution potential increased a little, the same as previous experiment, however, the dissolution current also increased. This indicated that more platinum in the system resulted in more silver dissolving. We can draw a conclusion that more platinum involved in the galvanic reaction taken place between pure silver and pure platinum did facilitate more silver dissolving. The absolute value of open circuit potential dropped with increasing the surface areas of the cathodic electrode of platinum.

**Figure 3.23** Galvanic study of silver dissolution between pure silver and pure platinum samples in 0.89wt% NaCl solution: pure silver (surface area: 0.785 cm$^2$); pure platinum with changing the surface areas from 0.00785 cm$^2$ (1m-Pt-Ag), 0.0157 cm$^2$ (2m-Pt-Ag) and 0.03925 cm$^2$ (5m-Pt-Ag).
Chapter 3 Silver Dissolution Behaviour from Silver and Silver-Platinum Alloys

\[
\text{AgCl} + e = \text{Ag} + \text{Cl}^-
\]

\[
2\text{H}_2\text{O} + \text{O}_2 + 4e = 4\text{OH}^-
\]

Figure 3.24 The model for the mixed potentials of silver dissolution between pure silver and pure platinum samples studied by zero resistance ammeter in 0.89wt% NaCl solution: pure silver (surface area: 0.785 cm\(^2\)); pure platinum with changing the surface areas from 0.00785 cm\(^2\) (1m-Pt-Ag), 0.0157 cm\(^2\) (2m-Pt-Ag) and 0.03925 cm\(^2\) (5m-Pt-Ag).
When silver and silver-platinum served as the anodes, with increasing platinum content in Ag-Pt alloys from 0% to 5%wt., the open circuit potential increased and the current density dropped. The model can be shown in Figure 3.22. This indicated that the increase of platinum in Ag-Pt alloys decreased the anodic electrode surface area. The effect of increasing of platinum on the surface area of the cathodic electrode of platinum (8 cm²) was so little that can be neglected.

**Figure 3.25** Open circuit potentials for Galvanic study of silver dissolution between platinum and silver-platinum alloys in 0.89wt% NaCl; 0PtAg, pure silver; 0.5PtAg, 0.5wt%Pt-Ag alloy; 1.5PtAg, 1.5wt%Pt-Ag alloy; 3PtAg, 3wt%Pt-Ag alloy; 5PtAg, 5wt%Pt-Ag alloy;
When the anode was pure silver and the cathode was pure platinum, the model of mixed potential for the effect of platinum on silver dissolution was shown in Figure 3.24. With increasing the cathodic surface area, the cathodic reaction was the driving force. The larger surface area of platinum involved, the more silver dissolved and the higher was the value of the potential.

**3.3.8 The effect of Platinum on silver dissolution behavior studied by Electrochemical Impedance Spectroscopy (EIS)**

The complex impedance and the bode impedance at pure silver and silver-platinum alloys, and 0.89%wt. NaCl solution was characterized by Electrochemical Impedance Spectroscopy (EIS) [27,28]. A sinusoidal signal of
10mV was superimposed on the signal of the open circuit potential. The data was collected between $10^4$ Hz and $10^2$ Hz. The complex impedance at pure silver or silver-platinum alloy and sodium chloride solution was shown in Figure 3.28 - Figure 3.31. The equivalent circuit model was shown in Figure 3.27. The impedance plot fitting data was listed in Table 3.7 and Table 3.8.

**Figure 3.27** Equivalent circuit model for silver or silver-platinum alloys sodium chloride solution interfaces.
Figure 3.28 Complex plane impedance plot for silver in 0.89%NaCl solution

Figure 3.29 Bode plane impedance plot for silver in 0.89%NaCl solution
Figure 3.30 Complex plane impedance plot for silver-platinum alloy in 0.89%NaCl solution

Figure 3.31 Bode plane impedance plot for silver-platinum alloy in 0.89%NaCl solution
In Figure 3.27, the equivalent model consisted an uncompensated solution resistance \( R_s \), a double layer capacitance \( C_{dl} \), a charge transfer resistance through the double layer \( R_{ct1} \), a film capacitance \( C_f \) and a charge transfer resistance related to the film \( R_{ct2} \). The capacitance was actually shown as a constant phase element (CPE). The unit for \( R_s \), \( R_{ct1} \) and \( R_{ct2} \) is Ohm cm\(^2\), and for \( C_{dl-T} \) and \( C_{f-T} \) is F cm\(^{-2}\) in Table 3.7 and Table 3.8.

For the double layer capacitance, the CPE-p values were 0.9041 for pure silver and 0.9093 for 5% wt. platinum alloy. This indicated a rough or porous surface caused a double layer capacitance to appear as a constant phase element with a CPE-p value between 0.9 and 1. The CPE was used in the model in place of a double layer capacitor to compensate for non-homogeneity in the system. The surfaces were shown very rough characterized by SEM in Figure 3.17 and 3.18.

Table 3.7 Pure silver sample fitting data of an equivalent circuit from EIS

<table>
<thead>
<tr>
<th>Element</th>
<th>Values</th>
<th>Error</th>
<th>Error %</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R_s )</td>
<td>61.3</td>
<td>0.0481</td>
<td>0.07887</td>
</tr>
<tr>
<td>( C_{dl-T} )</td>
<td>0.00012391</td>
<td>9.623E-7</td>
<td>0.77661</td>
</tr>
<tr>
<td>( C_{dl-P} )</td>
<td><strong>0.9041</strong></td>
<td>0.0014816</td>
<td>0.16388</td>
</tr>
<tr>
<td>( R_{ct1} )</td>
<td>5101</td>
<td>444.07</td>
<td>8.7055</td>
</tr>
<tr>
<td>( C_{f-T} )</td>
<td>0.00012195</td>
<td>1.2119E-5</td>
<td>9.9377</td>
</tr>
<tr>
<td>( C_{f-P} )</td>
<td><strong>0.58404</strong></td>
<td>0.030138</td>
<td>5.1603</td>
</tr>
<tr>
<td>( R_{ct2} )</td>
<td>9105</td>
<td>683.23</td>
<td>7.5039</td>
</tr>
</tbody>
</table>
Table 3.8 Silver-platinum alloy sample fitting data of an equivalent circuit from EIS

<table>
<thead>
<tr>
<th>Element</th>
<th>Values</th>
<th>Error</th>
<th>Error %</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_s$</td>
<td>60.56</td>
<td>0.04055</td>
<td>0.066958</td>
</tr>
<tr>
<td>$C_{dl-T}$</td>
<td>0.00014422</td>
<td>5.063E-7</td>
<td>0.35107</td>
</tr>
<tr>
<td>$C_{dl-P}$</td>
<td><strong>0.90932</strong></td>
<td>0.0008065</td>
<td>0.088693</td>
</tr>
<tr>
<td>$R_{ct1}$</td>
<td>14646</td>
<td>793.35</td>
<td>5.4168</td>
</tr>
<tr>
<td>$C_{f-T}$</td>
<td>0.00010792</td>
<td>8.5456E-6</td>
<td>7.9185</td>
</tr>
<tr>
<td>$C_{f-P}$</td>
<td><strong>0.5943</strong></td>
<td>0.031186</td>
<td>5.248</td>
</tr>
<tr>
<td>$R_{ct2}$</td>
<td>38413</td>
<td>3158.8</td>
<td>8.2233</td>
</tr>
</tbody>
</table>

For the capacitance related to a film of AgCl, the CPE-p values were 0.584 for pure silver and 0.594 for 5% wt. platinum alloy. These values were near to 0.5. A CPE with CPE-P value of 0.5 can be used to produce an Infinite Length Warburg element. A Warburg element occurs when charge carrier diffuses through a material. Hence, it can be deduced that the Ag$^+$ and Cl$^-$ diffuse through the silver chloride film. This has the same results compared to the work done by Bozzini and Payer [27-28].

Comparing the charge transfer resistance related to a double layer ($R_{ct1}$) and a film ($R_{ct2}$) for the pure silver and silver-platinum alloy in Table 3.7 and Table 3.8, the charge transfer resistance was much higher for silver-platinum alloy than that for pure silver. If the charge transfer resistance is higher, the kinetics for nucleation is slower and the reaction is delayed and occurred at higher potential.
This is one of the reasons why the potential where silver oxidizes for Ag-Pt alloys was higher than pure silver studied by cyclic voltammetry.

### 3.3.9 Mechanism of silver dissolution from silver and silver-platinum alloy in sodium chloride solution.

The mechanism of silver dissolution from silver and silver-platinum alloys in physiological solutions could be proposed as a double layer and a film mechanism based on literature [29-31], and this work of CV, SEM, EDAX and EIS measurement.

At an interface between the sample (working electrode) and the bulk solution containing chloride, during a potential sweep, at low potentials a double-layer charging current dominated initially. The electrical double layer resulted from adsorbed water and the reduction of oxygen that resulted in the specific adsorption of OH\(^-\) ions and the specific adsorption of Cl\(^-\) in saline solutions. Ag\(^+\) on the surface of silver or silver-platinum alloy sample diffused through the double layer and combined with the adsorbed Cl\(^-\) to form an AgCl film. Silver chloride formed and adsorbed on the surface of each sample. The following step was the nucleation of silver chloride and the formation of a thick silver chloride layer with channels and pores on the top of the surface. When the process reached a steady state, silver ions diffused through the porous AgCl layer.

In a chloride free solution, Ag\(^+\) diffused through the silver oxide layer into the bulk solution.
3.4 Conclusions

In the absence of chloride in the solution, silver dissolved to silver ions in an activation process and diffused away from the surface of the sample while with the presence of chloride, silver chloride was firstly formed in a diffusion process and it dominated the dissolution behaviour of silver from both pure silver and silver-platinum alloys.

For the range of alloys studied, there is no huge difference in the CV characteristics or the surface morphology. However, the nucleation of AgCl formed in the forward sweep process and the nucleation of Ag formed in the backward sweep process occurred for all samples but the addition of Pt inhibited the nucleation of AgCl and the formation of Ag⁺ in CV investigation. The AgCl grew on top of the sample and the reduced Ag formed a porous layer on top of Ag-Pt alloy. The layer of reduced Ag formed on Ag-Pt samples had a larger pore size than that formed on pure silver. The degree to which silver is oxidized, decreases with increasing platinum content both in absence and presence of chloride. The value of silver dissolution potential on fresh surface of each sample and the zero current potential increased with increase of level of Pt in the alloy.

ZRA results showed that platinum did enhance silver dissolution through a galvanic reaction between pure silver and pure platinum by increasing the amount of platinum involved.

With EIS measurement, an equivalent circuit model was setup for silver dissolution from silver and silver-platinum alloy in chloride solution: a double layer formed on the rough and porous surface of silver or silver-platinum samples;
a resistance produced by charge carrier transfer through the double layer; a layer of silver chloride formed by the diffused silver ions and the chloride ions, which produced a charge transfer resistance as well. The difference of silver dissolution behaviour between silver and silver-platinum alloy is the resistance produce from the charge carrier transfer that over silver-platinum alloy was much higher than over pure silver.
3.5 References


Chapter 4 Silver Dissolution from Polymers Plasma-Coated with Ag and Ag-Pt Alloys in Saline Solution
Abstract

Silver and silver platinum coatings deposited onto polyamide, polyethylene, poly vinyl chloride and silicone polymers by a combination of magnetron sputtering and atom beam sources. The dissolution behaviour of silver from these sputter-coated polymers was investigated by cyclic voltammetry in 0.89%wt. NaCl solution. The morphology of these sputter coated polymers was characterized by SEM.
4.1 Introduction

Infection is the most common serious complication of intravascular catheters. Most cases of catheter-related infection are caused by *staphylococci* that originate either from the skin of the patient and migrate along the external surface of the catheter or from a contaminated catheter hub and migrate along the internal surface of the catheter [1]. Silver coated urinary catheters have been studied in recent years to avoid the infection caused by catheters [1-8]. The anti-bacterial activity of silver is dependent on the silver cation (Ag$^+$), which binds strongly to electron donor groups on biological molecules containing sulfur, oxygen or nitrogen [9]. In order to enhance the anti-bacterial performance of silver, it is therefore necessary to increase the concentration of Ag$^+$ ions relative to that of metallic silver released from the coating. In the galvanic series silver is more active than platinum and therefore the latter metal should potentially enhance Ag$^+$ ion formation through galvanic action. There have been two patents [10,11] which have indicated the potential of Pt to enhance the level of Ag$^+$ ion formation in an alloy.

Silver and silver platinum coatings were deposited onto polyurethane and silicone polymers using a combination of magnetron sputtering and atom beam sources and their antibacterial activity was studied by Dowling et al [12-14]. The addition of 1% Pt significantly enhances the anti-bacterial effectiveness of the coating but does not appear to influence cytotoxicity. For the two polymers studied the cytotoxicity values were also dependent on the polymer type, the coating on silicone consistently giving lower cytotoxicity values than polyurethane.
This work is to study the silver dissolution behaviour on the silver and silver platinum plasma coatings deposited on polyamide, polyethylene, poly vinyl chloride and silicone and the effect of platinum on silver dissolution.

4.2 Experimental

4.2.1 Polymeric substrates

The polymer samples employed in this work are polyamide (PA), polyethylene (PE), polyvinylchloride (PVC), and silicone that are the products from Goodfellow Lmt.. The properties of these polymers are shown below:

Polyamide (PA) – Nylon 6,6 sheet
- Density: 1.14 g/cm³
- Flammability: HB
- Upper working temperature: 80-180 °C
- Limiting oxygen index: 23%
- Surface Energy (dynes/cm): <36

Polyethylene (PE) – Low density sheet
- Density: 0.92 g/cm³
- Flammability: HB
- Upper working temperature: 50-90 °C
- Limiting oxygen index: 17%
- Surface Energy (dynes/cm): 30.5

Polyvinylchloride (PVC) – Unplasticised sheet
Chapter 4 Silver Dissolution from Polymers Plasma-Coated with Ag and Ag-Pt Alloys in Saline Solution

Density: 1.4 g/cm$^3$

Flammability: VO

Upper working temperature: 50-75 °C

Limiting oxygen index: 42%

Surface Energy (dynes/cm): 33-38

Silicone Elastomer Sheet

Density: 1.1 – 1.5 g/cm$^3$

Upper working temperature: 200-260 °C

Surface Energy (dynes/cm): 24

4.2.2 Procedure and conditions for Ag and Ag-Pt alloy plasma coatings on polymers

The deposition of silver and silver-platinum alloy coatings on a polymer sheet was executed by magnetron sputtering combined with a neutral atom beam source (Figure 4.1) [13,14]. The advantage of this combination is that the metal can be deposited on a substrate at temperature of 70 °C rather than 200–300 °C as obtained by combining magnetron and radio frequency (RF) plasma (substrate holder bias) sources. The atom beam of argon plasma was applied at a current of 120 mA at 0.1 Pa both to activate the polymer surface for 60 s prior to coating deposition and during the silver and silver-platinum alloy deposition process. The samples were mounted approximately 35 cm from this source. Subsequent to
activation the magnetron shutter was opened and the silver and silver-platinum coatings were deposited at a deposition current of 0.4 A. The maximum deposition temperature that was measured by a thermocouple mounted alongside the polymer sample was 70°C. The deposition rate on a sample was 0.3nm/s on glass slides measured by glancing angle X-ray diffraction [13,14].

4.2.3 Electrochemical characterization of polymers sputtering-coated with silver and silver-platinum alloys

Cyclic Voltammetry (CV) experiment was carried out in a three-electrode cell connected to a Solartron 1287 potentiostat controlled with a Corrware software. The electrolyte was 0.89wt.% NaCl (SIGMA-ALDRICH) solution and oxygen
was removed by bubbling with nitrogen (BOC, 99.999%) before the measurement started. The reference electrode was a Saturated Calomel Electrode (SCE) and the counter electrode was a platinum foil with a surface area of 8 cm². The working electrode was a piece of polymer sheet plasma-coated with silver or silver-platinum alloy. The connection between the polymer sheet and the potentiostat was a conducting wire adhering with silver conducting glue on the polymer surface. The sweeping range was from -0.2 V to +0.1V vs. SCE and the scan rate was 10 mV/s.

4.2.4 Morphology characterization by SEM
The surface of each sample was characterized with Scanning Electron Microscopy (SEM, ZEISS Supra 35 VP) at different stages in the CV and the compositions of the surfaces were analyzed by EDX.

4.3 Results and Discussion

4.3.1 Silver dissolution from polyamide plasma-coated with Ag and Ag-Pt alloys

The dissolution behaviour of silver from the polymer of polyamide plasma-coated with silver and silver-platinum alloys was studied by cyclic voltammetry (CV) for three continuous cycles in 0.89% sodium chloride solution. The sweep range was from -0.2 to +0.1V vs. SCE. The scan rate was 10mV/s. Figure 4.2 shows the results of the first cycle of each samples. The pure silver coating gave the highest current density value at +0.1V vs SCE. For the second and the third cycles, less
silver dissolved. This was caused by the fact that less silver chloride reduced back
to silver on the polymeric polyamide. With increasing the amount of platinum in
the alloys coated on the polymer, less dissolution current density was produced
from the sample. This indicated there was less silver dissolved.

![Graph](image)

Figure 4.2 Silver oxidation from polyamide plasma-coated with Ag-5wt.%Pt
alloy studied by CV in 0.89wt.% NaCl solution from -0.2 to +0.1V vs. SCE,
scan rate: 10 mV/s.
Chapter 4 Silver Dissolution from Polymers Plasma-Coated with Ag and Ag-Pt Alloys in Saline Solution

The morphology of the surface on polyamide plasma coated with silver or silver-platinum alloys and of that measured by CV was characterized by SEM. All the samples showed similar morphology (Figure 4.3). A smooth surface produced...
with silver or silver-platinum alloy coatings. The surface after CV measurement produced some large circular patterns. The composition was measured by EDX. There was silver chloride formed. Therefore, silver chloride was formed after polarization of each sample in sodium chloride solution. In summary, the more platinum contained in the coatings on the polyamide, the less silver dissolved in chloride containing solution. The coating has very poor adhering activity to the surface of the polymer.

4.3.2 Silver dissolution from polyethylene plasma-coated with Ag and Ag-Pt alloys

Figure 4.4 shows the results of silver dissolution from polyethylene plasma-coated with pure silver and silver-platinum alloys (0.5 – 5%wt. Pt) studied by CV in 0.89wt.% NaCl solution from -0.2 to +0.1V vs. SCE with a scan rate of 10 mV/s. It appears that the sample coated with 3%wt.Pt alloy showed the highest current density at +0.1 V vs. SCE which was similar to that over the sample with pure silver coatings. The rest of the samples coated with silver-platinum alloys showed lower current density. The more platinum contained, the less silver dissolved. A more smoother surface on polyethylene coated with silver or silver-platinum alloy was produced compared to those on polyamide studied by SEM (Figure 3.5). After analysing the composition of the surface, there was almost no silver chloride from the oxidation of silver on the surface after CV measurement (Figure 4.5).
Chapter 4 Silver Dissolution from Polymers Plasma-Coated with Ag and Ag-Pt Alloys in Saline Solution

Figure 4.4 Silver dissolution from polyethylene plasma-coated with Ag-5wt.%Pt alloy studied by CV in 0.89wt.% NaCl solution from -0.2 to +0.1V vs. SCE, scan rate: 10 mV/s.
Figure 4.5 Morphology of polyethylene plasma-coated with silver studied by CV from -0.2 to +0.1 V vs. SCE in 0.89% NaCl solution, characterised by SEM (top) and its surface compositions measured by EDX (bottom)
4.3.3 Silver dissolution from poly vinyl chloride plasma-coated with Ag and Ag-Pt alloys

For the samples of poly vinyl chloride plasma coated with pure silver or silver-platinum alloys, Figure 4.6 showed the results of silver dissolution studied by CV in 0.89wt.% NaCl solution from -0.2 to +0.1V vs. SCE with a scan rate of 10 mV/s. The sample of pure silver coatings showed the least current density at +0.1 V vs. SCE. With more platinum contained in the alloys, less silver dissolved. The morphology in Figure 4.7 showed that there were very large particles produced from the pure silver or silver-platinum alloy coatings. There was a huge amount chloride on the surface. This might result from the polymer of PVC or the formation of silver chloride.

![Graph showing silver dissolution from poly vinyl chloride plasma-coated with Ag and Ag-Pt alloys](image)

Figure 4.6 Silver dissolution from poly vinyl chloride plasma-coated with Ag-5wt.%Pt alloy studied by CV in 0.89wt.% NaCl solution from -0.2 to +0.1V vs. SCE, scan rate: 10 mV/s.
Figure 4.7 Morphology of poly vinyl chloride plasma-coated with silver studied by CV from -0.2 to +0.1 V vs. SCE in 0.89% NaCl solution, characterised by SEM (top) and its surface compositions measured by EDX (bottom)
4.3.4 Silver dissolution from silicone plasma-coated with Ag and Ag-Pt alloys

Silver dissolution behaviour from silicone plasma coated with silver or silver-platinum alloy studied by CV in 0.89%wt. NaCl solution was shown in Figure 4.8.

There was some species already oxidized before silver was oxidized. The rate for silver dissolution was higher over the silver-platinum coatings than that over the pure silver coating.

The morphology in Figure 4.9 showed that the surface was significant rougher compare to those surfaces of PA, PE and PVC. There was a huge amount chloride contained on the surface (Figure 4.9 EDX) even it was washed with deionised water after CV measurement.

![Graph](image)

**Figure 4.8** Silver dissolution from silicone plasma-coated with Ag-5wt.%Pt alloy studied by CV in 0.89wt.% NaCl solution from -0.2 to +0.1V vs. SCE, scan rate: 10 mV/s.
Figure 4.9 Morphology of silicone plasma-coated with silver studied by CV from -0.2 to +0.1 V vs. SCE in 0.89% NaCl solution, characterised by SEM (top) and its surface compositions measured by EDX (bottom)
4.3.5 The effect of the type of polymer on silver dissolution from Ag and Ag-Pt alloys targets

The dissolution behaviour of silver from pure silver and silver-platinum alloy targets was studied by cyclic voltammetry (CV) in 0.89% sodium chloride solution in the same sweep range as those polymers as -0.2 to +0.1V vs. SCE. The scan rate was 10mV/s. Figure 4.10 shows the results. Pure silver sample gave the highest current density while the silver-platinum alloy sample gave less current density with decreasing amounts of platinum. The potential for silver started to dissolve with increase in the amount of platinum alloy as well.

![Graph showing silver dissolution from Ag and Ag-Pt alloys](image)

Fig. 4.10 Silver dissolution from silver and silver-platinum alloys studied by CV in 0.89wt.% NaCl solution from -0.2 to +0.1V vs. SCE, scan rate: 10 mV/s.
Chapter 4 Silver Dissolution from Polymers Plasma-Coated with Ag and Ag-Pt Alloys in Saline Solution

We compared the silver dissolution behaviour from pure silver to that coated on poly amide (PA), poly ethylene (PE), poly vinyl chloride (PVC) and silicone. The line in black color in each graph was the first cycle for each sample. It shows the obvious difference for silver dissolution from each polymer. The CV behaviour of silicone sample was very different from the others. Silver started dissolving significantly from 0.05 V vs. SCE over the samples of PA, PE and PVC while oxidation process already took place in a negative range (-0.2V vs. SCE) in the forward sweeping process in CV.

![Bar chart showing silver dissolution from different polymers](chart.png)

Fig. 4.11 the potential for silver dissolution from silver and silver-platinum alloys studied by CV in 0.89wt.% NaCl solution from -0.9 to +0.1V vs. SCE, scan rate: 10 mV/s.

Compared to the coating with 0.5% Pt-Ag alloys and 5% Pt-Ag alloys on PA, PE, PVC and silicone, there were some changes from those pure silver coatings. PA and PE samples had similar behaviour: pure silver coating gave the highest current; PVC samples pure silver coating sample gave the lowest dissolution current; For silicone coating samples, the alloys gave higher current than pure silver coatings.
Comparing the dissolution behaviour of silver from the silver metal and silver-platinum alloys and from the polymers, pure silver sample gave the highest dissolution current and with increasing the amount of platinum in the alloys, the dissolution current dropped. In another words, the more silver contained in the alloys, the more silver dissolved from the alloys. The potential where silver starting to dissolve was higher over pure silver samples than silver-platinum alloy samples for target and the polymers, except for silicone sample. It is shown in Figure 4.11. Figure 4.12 showed the zero current potential for silver dissolution from silver and silver-platinum alloys studied by CV in 0.89wt.% NaCl solution from -0.9 to +0.1V vs. SCE, scan rate: 10 mV/s.
the target samples and the PVC samples plasma coated with silver or silver-platinum alloy. Over the rest samples, there was no clear trend.

Comparing the morphology of the polymers plasma coated with silver on PA, PE, PVC and silicone, the coatings on silicone showed some large while the other three were more homogeneous.

4.4 Conclusions

The dissolution behaviour of silver from the plasma-coated polymers with Ag and Ag-Pt alloys depends on the properties of the polymers (PA, PE, PVC and silicone). Under the condition with the presence of chloride, AgCl formed and peeled away from the polymers.

The morphology of the coatings depends on the type of polymers as well. The coating on silicone sheets showed the metals existing as irregular particles while the others were in a homogeneous state. The results indicate that the sputtering was successful in coating the platinum with the silver. Such small platinum levels can be difficult to measure by EDX.
4.5 References

Chapter 5 Silver Dissolution Behaviour from a Polymeric Silver Nanoparticle Composite
Abstract

Silver nano-particles stabilized with a series of polymeric composites were prepared by the reduction of AgNO$_3$ with NaBH$_4$ under the protection of polymers. The silver nano-particles had a broad absorption band at 390-420 nm measured by UV-vis spectrophotometer. The absorption band shifted with changing the polymer stabilizer. Silver nano-particles were characterized by XRD and TEM as well. The stability of silver nano-particles was dependent on the properties of each polymer. Silver dissolution behaviour from polymeric silver nanoparticle composite was investigated by Cyclic Voltammetry in 0.89%wt. NaCl saline solution.
Chapter 5 Silver Dissolution Behaviour from a Polymeric Silver Nanoparticle Composite

5.1 Introduction

One of the most important biomedical applications of silver is silver coatings used for medical devices [1-5] to reduce infections related to surgery, for example, catheters-related infections in hospitals [1-3,6-11], and silver containing wound dressings [12-14]. Metallic silver has only a slight antibacterial effect because it is chemically stable but silver ions are active against a broad spectrum of bacteria with the antimicrobial activity being proportional to the free silver ions concentration [4,8,12,15-17]. However, in an environment containing albumin and halide ions, the antibacterial activity of silver ions will be decreased as a result of specific absorption process with albumin and precipitation into insoluble silver chloride. This is one of the reasons that silver ions cause the cytotoxicity as well.

The attention of many scientific groups has been drawn to the area of silver nanoparticles, for example, the Toshima research group has prepared and studied silver nano-particles used as catalysts for oxidation of ethylene [18-23], as well as the research group of Jong Hak Kim [24-26]. Recently silver nano-particles combined with polymers have been studied for their applications as antibacterial materials [27,28].

A summary of silver nanoparticles prepared by different chemicals and methods, and their applications is listed in Table 5.1.

This work involved in the preparation of polymeric composites combined with silver nanoparticles and investigated silver dissolution behaviour from the polymeric composite by Cyclic Voltammetry in 0.89%wt. NaCl solution.
Chapter 4 showed that thin silver coatings on polymeric materials had very poor adherence. Therefore, this work aimed to prepare novel polymeric materials containing silver nanoparticles to improve the delivery of silver ions for antibacterial purposes.
### Table 5.1 The review of preparation of silver nano-particles

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Preparation</th>
<th>Applications</th>
<th>Advantage and disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Silver salts:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silver 2-ethylhexanoate [Ag(ethex)]</td>
<td>0.00125g of Ag(ethex) was mixed with 50 mL of DMSO in an Erlenmeyer flask</td>
<td>The nano-particles of silver were prepared as a catalyst for the reduction of NO.</td>
<td>The size of NPs in 4-10nm.</td>
</tr>
<tr>
<td>Silver nitrate (AgNO₃)</td>
<td></td>
<td></td>
<td>Process is simple.</td>
</tr>
<tr>
<td>Silver citrate hydrate</td>
<td></td>
<td></td>
<td>DMSO, Garlic odor</td>
</tr>
<tr>
<td>Silver perchlorate monohydrate</td>
<td></td>
<td></td>
<td>Avoid lights</td>
</tr>
<tr>
<td>Silver metavanadate (AgVO₃)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduction agents and stabilizers:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMSO (dimethyl sulfoxide)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium borohydride NaBH₄</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trisodium citrate dehydrate Na₃Cit.2H₂O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium 2-ethylhexanoate Na(ethex)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chemicals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colloidal Ag-NPs (0.054%, average</td>
<td>Colloidal NP</td>
<td>Ag-NPs has the antibacterial activity but Pt-NP has no antibacterial activity.</td>
<td>PVP is more active than SDS.</td>
</tr>
<tr>
<td>diameter: 10 nm) solution and Pt-NPs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(0.054%, average diameter: 3 nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>solution containing poly-(N-vinyl-2-pyrrolidone) (PVP) and sodium dodecylsulfate (SDS) as a</td>
<td></td>
<td></td>
<td>5ppm Ag-NP-PVP is not enough to kill bacteria. 10-20ppm killed 99% and 100ppm killed all.</td>
</tr>
</tbody>
</table>
stabilizer were prepared using ultrasonic irradiation method. | | | The wall of the cell damaged in Ag-NP medium investigated by SEM

<table>
<thead>
<tr>
<th>Title</th>
<th>A novel single step chemical route for noble metal nanoparticles embedded organic-inorganic composite films [31] 2005 India</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals</strong></td>
<td><strong>Preparation</strong></td>
</tr>
<tr>
<td>Sol-gel preparation For a typical synthesis, 1.5 g of polyvinyl alcohol (PVA72000) was dissolved in 30 ml distilled water stirred for 30 min and 2.5 ml of tetraethyl orthosilicate (TEOS) was added and the mixture was stirred for 10 min, then 0.5 g of silicotungstic acid (SiW) was added and refluxed at 343K for 6 h. The obtained gel was coated on glass slides by spin coating method and dried at room temperature.</td>
<td>Sol-gel + reduction by sunlight Another paper</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Title</th>
<th>Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria [27] 2004 Croatia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals</strong></td>
<td><strong>Preparation</strong></td>
</tr>
<tr>
<td>Silver NP was obtained by precipitation method.</td>
<td>Silver hydrosols prepared in ascorbic acid solution, followed by washing and by</td>
</tr>
</tbody>
</table>
### Chapter 5 Silver Dissolution Behaviour from a Polymeric Silver Nanoparticle Composite

<table>
<thead>
<tr>
<th>Title</th>
<th>Prevention of catheter-related infections: the potential of a new nano-silver impregnated catheter</th>
<th>[32] 2004 Germany</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals</strong></td>
<td><strong>Preparation</strong></td>
<td><strong>Applications</strong></td>
</tr>
<tr>
<td>The even distribution of 10^{12-13} activated Ag-NP per gram in various polymers: polyurethane and silicone.</td>
<td>Catheter was impregnated with silver NP.</td>
<td>Another paper</td>
</tr>
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<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Title</th>
<th>Synthesis of silver/polymer colloidal composites from surface-functional porous polymer microspheres</th>
<th>[28] 2004 Korea</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals</strong></td>
<td><strong>Preparation</strong></td>
<td><strong>Applications</strong></td>
</tr>
<tr>
<td>Ag^+ + N_2H_4+4OH^- = 4Ag+4H_2O+N_2</td>
<td>Deposition of Ag-NP on polymers</td>
<td>centrifuging</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Title</th>
<th>Nano-cluster catalyse...</th>
<th>[18-23] Naoki Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals</strong></td>
<td><strong>Preparation</strong></td>
<td><strong>Applications</strong></td>
</tr>
<tr>
<td>Polymers stabilize metallic nanoclusters</td>
<td>Polymer solved in silver salt (silver perchlorate) alcohol and water solution in Nitrogen protection. Heating to 90-95 °C or by UV (refluxing).</td>
<td>Applied for oxidation of ethylene (catalysts)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Title</th>
<th>Poly N-vinyl pyrrolidone</th>
<th>[24-26] Korea</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals</strong></td>
<td><strong>Preparation</strong></td>
<td><strong>Applications</strong></td>
</tr>
<tr>
<td>Poly N-vinyl pyrrolidone</td>
<td>Polymer – silver</td>
<td>Membranes catalysts for</td>
</tr>
</tbody>
</table>

Freeze drying.
### Chapter 5 Silver Dissolution Behaviour from a Polymeric Silver Nanoparticle Composite

<table>
<thead>
<tr>
<th>Title</th>
<th>Self-assembly of silver nanoparticles in a polymer solvent: formation of a nanochain through nanoscale soldering</th>
<th>2005</th>
<th>Africa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals</strong></td>
<td>Methoxy polyethylene glycol-5000 and silver nitrate</td>
<td><strong>Preparation</strong></td>
<td>Photochemical reaction With nitrogen gas purged to remove dissolved oxygen.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Title</th>
<th>In-situ formation of Ag-containing nanoparticles in thin polymer films</th>
<th>1998</th>
<th>Germany</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemicals</strong></td>
<td>Polyvinylalcohol (PVOH) and polyvinylpyrrolidone PVP Silver nitrate</td>
<td><strong>Preparation</strong></td>
<td>Photochemical reaction</td>
</tr>
</tbody>
</table>

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5.2 Experimental

5.2.1 Preparation of polymeric silver nano-particle composites

5.2.1.1 Chemicals

Silver nitrate AgNO$_3$ (Aldrich)

Sodium borohydride NaBH$_4$ (Aldrich)

Ethanol CH$_3$CH$_2$OH (Aldrich)

Tetrahydrofuran (CH$_2$)$_4$O (THF, Aldrich)

Poly vinyl alcohol (C$_2$H$_4$O)$_n$ (PVA, MERCK)

Poly ethylene glycol (PEG, 20000, Fluka)

Poly vinyl chloride (CH$_2$CHCl)$_n$ (PVC, Aldrich)

Poly vinyl pyrrolidone (C$_6$H$_9$NO)$_n$ (PVP, Aldrich)

Adipic acid C$_6$H$_{10}$O$_4$
164

Chapter 5 Silver Dissolution Behaviour from a Polymeric Silver Nanoparticle Composite

5.2.1.2 Procedure

0.22g poly vinyl alcohol (PVA, MERCK) was dissolved into solution of distilled water and ethanol (Aldrich) (1:1) at 80 °C. The desired amount of 0.01M silver nitrate (Aldrich) aqueous solution was added into the PVA solution at room temperature. The silver nano-particles stabilized by PVA were obtained by introducing sodium borohydride (Aldrich) aqueous solution. The colour of the solution suddenly changed to dark-brown indicated the formation of silver nano-particles (Figure 5.1). The silver nano-particles stabilized with PVA were crosslinked by adipic acid under proper conditions.
Nano-silver particles stabilized in ploy ethylene glycol (PEG, 20000, Fluka) and poly vinyl pyrrolidone (PVP, Aldrich) were prepared by the same method described above.

Silver nano-particles stabilized with poly vinyl chloride (PVC, Aldrich) were prepared in THF (Aldrich) solution and ethanol solution (1:1 mixture). The procedure was the same as for preparation silver nano-particles stabilized with PVA. The only change was the solution of THF because PVC dissolves in THF and not in water.

5.2.2 Characterisation of polymeric silver nano-particle composites
The polymeric silver nano-particle composite was characterized by a UV-vis spectroscopy (UV-210PC, MASON Technology), X-Ray Diffraction (XRD, SIEMENS DIFFRAKTOMETER), Transmission electron microscope (TEM,
JEOL, J1000CX), SEM and EDAX, and Raman Spectroscopy (Jobin Yvon) techniques.

Cyclic Voltammetric (CV) experiments were carried out in a three-electrode cell connected to a Solartron 1287 potentiostat controlled with Corrware software. The reference electrode used throughout the investigation was a Saturated Calomel Electrode (SCE). The counter electrode was a platinum wire. A glassy carbon electrode coated with polymeric silver nanoparticle composite served as the working electrodes.
5.3 Results and Discussions

5.3.1 Silver nano-particles stabilized with polymers characterized by UV vis spectroscopy

Figure 5.2 UV-vis spectra for the samples containing Ag nano-particles: a. Ag-PVA prepared after 2 hours; b. Ag-PVA prepared after 12 days; c. Ag-PVA prepared after 35 days; d. Ag-PVA crosslinked with adipic acid cast on a microscope slide

The process of preparation of silver nano-particles was described in experimental part. When the colour of the solution suddenly changed to dark redish brown, it indicated the formation of the nano-particles in the polymer solution. The solution after dilution in ethanol was characterized by UV-vis spectroscopy and the spectra of nano-silver particles stabilized with PVA were shown in Figure 5.2. The peak
at ca. 390-400 nm showed the absorption spectra typical of silver nano-particles at room temperature and exposed to air. Curves a - c in Figure 5.2 indicated that the silver nano-particles were stable even after exposed to air after 35 days. Silver nano particles stabilized with PVA are water soluble and do not form stable films in aqueous solution. To decrease the solubility of the composite of silver nano particle stabilized with PVA, the composite was crosslinked with adipic acid. The rate of dissolution for the composite was slowed down. Curve d in Figure 5.2 showed a film of silver nano-particles were still stable after crosslinking PVA with adipic acid under proper conditions.

Figure 5.3 UV-vis spectra for the samples containing colloidal Ag nano-particles stabilized with PVP (poly vinyl pyrrolidone)
Figure 5.3 shows the characterization of nano-silver particles stabilized in PVP (poly vinyl pyrrolidone) by UV-vis spectrometer. There was a small peak at 330-350nm. This indicates that nanoparticles in PVP were much smaller than silver nanoparticles in PVA. The colour of the solution showed dark-blue or grey. After one week, there was mirror effect formed on the surface of the container (flask). It was characterized by XRD, there’s no silver crystals formed (brown line in Figure 5.5).

Figure 5.4 is the spectra of silver nano-particles stabilized in PVC (poly vinyl chloride). It shows a very wide peak at 420-450 nm. The colour of the solution was yellowish. There has been very little work using PVC as stabilising agent. An advantage of the system is that THF solutions are easier to cast into uniform films than water.
The silver nano-particles stabilized in PEG (poly ethylene glycol) were very unstable and there was large black particles precipitated. It’s hard to characterise them by UV-vis spectrometer after preparation.

Khanna [35] prepared Ag-PVA nanocomposite by chemical reduction method. The absorption band of the silver nanocomposite, which was reduced by sodium formaldehyde sulfoxylate, was at 418 nm. The absorption bands at 425-435 nm were observed by Fritzsche[34] by preparing silver containing nanoparticles in thin polymer films (poly vinyl alcohol and poly vinyl pyrrolidone). The absorption bands of silver nano-particles shifted to around 400 nm because the size and the distribution in size of silver nano-particles were different to each samples prepared by different ways [26].

5.3.2 Silver nano-particles stabilized with polymers characterized by XRD
The samples prepared in this work were characterized by XRD to confirm the existence of silver nano-particles stabilized with PVA, PVP, PVC and PEG. The results were shown in Figure 5.5. The peaks sitting at 38.5 degrees, 44.5 degrees, and 65.5 degrees showed (111), (200), and (220) diffraction of face centered cubic (fcc) silver crystals, respectively [28,33]. These peaks were not very sharp because the crystals of silver nanoparticles did not grow into good shape crystalline or were very small size. The PEG sample in figure 5.5 showed a very sharp and strong peak at about 33 degrees. This indicates the formation of larger crystallites that were caused by the non-stability or agglomerate of silver oxide compound in PEG.
5.3.3 Silver nano-particles stabilized in PVA characterized by TEM

The morphology of silver nano-particles stabilized with PVA was investigated by TEM. Figure 5.6 shows the TEM image of silver nano-particles stabilized PVA crosslinked by adipic acid after dilution in ethanol at room temperature. The silver nano-particles were cast in the PVA polymer film and it can be seen that there are some pores left in the polymer film. These pores appeared to be like the gaps left by silver nano-particles lost from the polymer or produced by solvent evaporating form the polymeric materials.
5.3.4 Silver nano-particles stabilized in PVA characterized by Raman Spectroscopy

Surface Enhanced Raman Spectroscopy (SERS) was measured with a helium-neon laser source of 514 nm for silver nanoparticle polymeric composites. The spectra are shown in Figure 5.7. Line (a) shows the spectrum of the sample of polymer PVA while line (b) for silver nanoparticle stabilized with PVA and line (c) for silver nanoparticles stabilized with PVA crosslinking with poly vinyl pyridine and dibromohexane, respectively. There was no obvious adsorption peak over the sample of PVA. Two broad bands appearing around 1143.8 cm\(^{-1}\) and 1349.3 cm\(^{-1}\) for the sample of silver nanoparticles stabilized with PVA were caused by Ag nano-particles SERS enhancement. When silver nanoparticle stabilized with PVA crosslinking with poly vinyl pyridine and dibromohexane, the peak sitting at around 1143.8 cm\(^{-1}\) became stronger in intensity while the peak sitting at around 1349.3 cm\(^{-1}\) became weaker and became a shoulder.
Endrino et al. [36] studied the film plasma coated with silver by Raman Spectroscopy and found there were two bands sitting at around 1350 cm\(^{-1}\) and 1530 cm\(^{-1}\) contributed to interaction between silver and polymers. Williams et al [37] suggested that the band at 1040 -1150 cm\(^{-1}\) was due to C-O stretching bond and the band at 1260-1410 cm\(^{-1}\) contributed to O-H bending bond in RCOH. This has the same agreement in our results shown in Figure 5.7. The introducing of silver nanoparticles in PVA caused the SERS enhancement of the C-O stretching bond at 1143.8 cm\(^{-1}\) and the O-H bending bond at 1349.3 cm\(^{-1}\) in PVA (Figure 5.7 spectrum b). After crosslinked Ag nanoparticles-PVA with dibromohexane and poly vinly pyridine, the band at 1349.3 cm\(^{-1}\) due to O-H bending bond became
weaker and formed a shoulder (Figure 5.7 spectrum c). This is caused by the loss of O-H that involved the formation of H$_2$O during the crosslinking process, which was removed from the crosslinked polymeric composite.

5.3.5 Silver dissolution from Ag nano particles composite with polymers in 0.89%NaCl solution

Silver nanoparticles stabilized with PVA were crosslinked with polv vinyl pyridine (PVP) and dibromohexane cast on a glassy carbon electrode at 60 °C. The morphology was characterized by SEM (Figure 5.8) and the composition was measure by EDAX (Figure 5.9). The sample was coated with gold in a sputter chamber to produce a conductive surface on the polymeric substrate. The left photo in Figure 5.8 showed a mixture of some large particles and a fabric texture substrate. The large particles were identified as silver particles by EDX. The composition of the fabric texture substrate was analyzed by EDX and the results were shown in Figure 5.9. The signal of silver was a little bit stronger than that of carbon in Figure 5.9. The silver nanoparticles were probably supported in the fibres. From Figure 5.9 it can be seen that there are dominant signals from silver and sodium also indicating high silver loading.
The crosslinked polymeric substrate containing silver nanoparticles was cast on a glassy carbon electrode. Silver dissolution behaviour was investigated by cyclic voltemmetry in 0.89%wt. NaCl solution (Figure 5.10). The scan range was from -
0.4V to +0.2V vs. SCE and the scan rate was 10mV/s. Silver started dissolving at the potential of ca. 0.070V vs. SCE and reached the maximum dissolution rate at +0.095V (Figure 5.10 A). This potential value is higher than silver started to dissolve from pure silver target shown in Chapter 3 and that for silver started to oxidize from a polymeric substrates (PA, PE and PVC) plasma coated with pure silver shown in Chapter 4. On the backward sweep, silver was completely reduced at potential of -0.090V vs. SCE and reached the reduction peak at the potential of -0.065V. The potential value of silver reduction peak was more negative than those formed by the polymeric substrates plasma coated with pure silver shown in Chapter 4. For the three continuous cycles measured in this work, the silver oxidized to silver chloride and reduced back. There was almost no silver lost in the solution during repetitive cycling in the CV investigation. This shows the stability of the silver in the film unlike the plasma coated films that peeled away on scanning.

It can be seen that there are two couples (Figure 5.10 B); the first was oxygen reduction and the second was the Ag/AgCl couple.
Figure 5.10 CV results of polymeric silver nano-particle composite in 0.89%wt. NaCl solution; sweep range: -0.4 to +0.2 V vs. SCE; scan rate 10 mV/s; top, I vs E; bottom, lgI vs E.
5.4 Conclusions

Silver nano-particles were prepared and stabilized in polymers (PVA, PVP, PVC and PEG) by the reduction of silver nitrate with sodium borohydride. The stabilities and morphology is dependant to the properties of the polymers: PVA and PVC showed good stability to the silver nanoparticles, while silver nanoparticles PVP and PEG were unstable samples in this work. The size and the distribution in size of silver nano-particles was also dependant on the type of polymers. This was confirmed by results of UV vis spectra, XRD patterns and TEM images of silver nano-particles.

SERS spectra showed two bands sitting at around 1143.8 cm\(^{-1}\) and 1349.3 cm\(^{-1}\) enhanced by the silver nanoparticles.

The dissolution of silver from silver nanoparticle polymeric composite studied by CV in sodium chloride solution had the similar behaviour as that of pure silver or the samples of polymer plasma coated with silver. The advantage of silver nanoparticle polymeric composite is the reproducibility of oxidation and reduction of silver to silver chloride and stability of the silver nanoparticle polymeric composite compared to plasma coated polymeric material.
Chapter 5 Silver Dissolution Behaviour from a Polymeric Silver Nanoparticle Composite

5.5 Reference


Chapter 5 Silver Dissolution Behaviour from a Polymeric Silver Nanoparticle Composite

Chapter 5 Silver Dissolution Behaviour from a Polymeric Silver Nanoparticle Composite


Chapter 6 The Antibacterial Activity of Silver Nano-particles Stabilized with PVA
Abstract

The antibacterial activities against strains of *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) of silver nano-particles composite were investigated. Silver nanoparticles stabilized with PVA crosslinked by adipic acid showed strong antibacterial activities against both *E. coli* and *S. aureus*. The Alamar Blue assay and the Neutral Red assay were employed to assess metabolic activity, the membrane function and lysosomal activity of A549 cells to evaluate the cytotoxicity the silver nanoparticle composite
6.1 Introduction

Silver has been drawn to the attention to scientists used as a biomedical material because of its antibacterial activities [1-6]. Nanoparticles exhibit novel material properties that largely differ from the bulk materials due to the small sizes, including quantum size effect in photochemistry [7-12]. Synthesis of silver nanoparticles is of a great interest to scientists for the applications used as antibacterial materials, antistatic materials, cryogenic superconducting materials and biosensor materials [2,13-16].

The antibacterial function of silver nanoparticle materials is one of the key parameters to the success of their applications. A large number of methods were developed worldwide to examine the relationship between microorganisms and the materials [17]. JIS Z 2801:2000 was a method to test the antibacterial activity and efficacy for antimicrobial products proposed by scientists in Japan and contributed to the development of the ISO/NP 22196 standard for testing antibacterial activity [18].

Due to the great interest in developing novel applications of nanomaterials, the evaluation of toxicity of the nanomaterials has been carried out in vitro studies based on cell cultures, such as the test of cytotoxicity for single wall carbon nanotubes [19-24]. The Alamar Blue (AB) assay evaluates the metabolic activity of cells based on the reduction of resazurin (blue and nonfluorescent) to resorufin (pink and highly fluorescent) in the presence of metabolically active cells [25]. The Neutral Red (NR) assay has, in many instances, been used for the quantification of cytotoxicity based on the ability of viable cells to incorporate and accumulate the weakly cationic supravital dye within lysosomes [1,21,26,27]. These assays provide information about basal cytotoxicity and reflect the intrinsic
ability of test compounds to cause cell death as a consequence of damage to basic cellular functions.

This work focuses on the measurement of the antibacterial activity of silver nanoparticles stabilized in poly vinyl alcohol (PVA) prepared in Chapter 5 for the potential application as a novel biomaterial. The cytotoxicity of silver nanoparticles stabilized in PVA was also evaluated by the methods of AB and NR assays and preliminary results are shown here.

6.2 Experimental

6.2.1 Preparation and characterisation of silver nanoparticles

Silver nanoparticles stabilized with poly vinyl alcohol (PVA, from MERCK) were prepared by reduction of silver ion under the protection of PVA. The method was described in Chapter 5. The silver nano-particles stabilized with PVA were crosslinked by adipic acid under proper conditions. The silver nano-particles stabilized with PVA (Ag-NP/PVA) were characterized by UV-vis Scanning Spectroscopy (UV-210PC, MASON Technology), XRD (SIEMENS DIFFRAKTOMETER), and Transmission electron microscope (TEM, JOEL) instruments, respectively.
6.2.2 Antibacterial activity testing

6.2.2.1 Culture Media

Mueller-Hinton Agar is a major media used for Antimicrobial Susceptibility Testing (AST). It was designed to be a reproducible culture medium for the isolation of pathogenic Neisseria species. CM337 Mueller-Hinton Agar employed in this work to assess the antibacterial activity of the silver nanoparticle polymeric composites prepared in this work. The property and composition of Mueller-Hinton Agar CM337 are listed in Table 6.1

Maximum Recovery Diluent (MRD, CM733, Peptone saline diluent) combines the protective effect of peptone in the diluting solution with the osmotic support of physiological saline. The compositions of the MRD CM733 show in Table 6.1.

The culture media were prepared in distilled water and sterilized by autoclaving 121 °C for 15 minutes.

Table 6.1 The compositions of the culture media used in this work

<table>
<thead>
<tr>
<th></th>
<th>Mueller-Hinton Agar CM337</th>
<th>Mueller-Hinton Broth CM405</th>
<th>Maximum Recovery Diluent CM733</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef, dehydrated infusion form</td>
<td>300.0</td>
<td>300.0</td>
<td>Peptone 1.0</td>
</tr>
<tr>
<td>Casein hydrolysate</td>
<td>17.5</td>
<td>17.5</td>
<td>Sodium chloride 8.5</td>
</tr>
<tr>
<td>Starch</td>
<td>1.5</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>17.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.4±0.2</td>
<td>7.3±0.1</td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>
6.2.2.2 Antibacterial activity test procedure

The antibacterial activity of the silver nano-particles stabilized with PVA was measured against the Gram negative bacteria of *E. coli* (10418 for sensitive test) and the Gram positive bacteria of *S. aureus* (25923 for sensitive test), respectively.

MUELLER-HINTON agar (CM337 OXOID) was used as the culture medium for the antibacterial susceptibility testing (AST) in an internationally recognised standard procedure [28]. The silver nano-particles stabilized with PVA was introduced into a well (diameter: 6mm) in the centre of the culture agar medium. The agar medium was incubated at 37 °C for 20 hours.

Maximum Recovery Dilute (MRD) measurement [29] was carried in MRD agar (CM733 OXOID, peptone saline diluent) for testing the efficiency of silver nano-particles stabilized with PVA on antibacterial activity. The original solution of silver nano-particles stabilized with PVA was diluted to half concentrations of previous one in the MRD medium until the medium lost the antibacterial activity. The MRD medium was incubated at 37 °C for 20 hours.

The effect of the silver nano-particles stabilized with PVA on the bacteria was evaluated after MRD measurement. The test was performed by taking some specimens from the medium with antibacterial active action and re-culturing in the medium for bacterial growing.
6.2.3 In vitro cytotoxicity test

Alamar Blue™ (AB) cell culture media and supplements and the trypsinisation solution were purchased from Biosciences (Dublin, Ireland). Neutral Red (NR) dye was purchased from Sigma-Aldrich (Ireland).

The human alveolar carcinoma epithelial cell line A549 (ATCC, CCL-185) was employed for toxicity evaluation. Cells were grown in F-12K medium (Kaighn’s Modification, Gibco) supplemented with 10% foetal calf serum (FCS), 45 IU ml\(^{-1}\) penicillin and 45 µg ml\(^{-1}\) streptomycin and grown in a humidified incubator at 37°C (5% CO\(_2\)) [23,30,31].

Individual wells of a 96 well microplate (Nunc, Denmark) were seeded with 100 µl of cell suspension containing 1x10\(^5\) cells/ml for cytotoxicity tests. A stock concentration of 0.002 M solution of silver nanoparticles stabilized with PVA was prepared in a 20% ethanol solution. A 1:10 working solution was prepared from this stock in cell culture medium supplemented with 5% foetal bovine serum and this concentration was then serially diluted with culture medium to prepare a test concentration range of 0.003-0.2 µM. A solvent control which contained the highest concentration of a solution without silver nanoparticles used in the test was incorporated in each test. Chemical exposure was conducted for 24 h. After this incubation period, the test medium was removed, cell monolayers washed with phosphate buffered saline (PBS) and prepared for cytotoxicity assays.

Viability of the cells following exposure to the silver nanoparticle composite was evaluated using the AB and NR assays. The AB and NR assays were conducted subsequently on the same set of plates. The AB assay was performed first. The bioassay was carried out according to manufacturer’s instructions. Briefly, control
media or test exposures were removed; the cells were rinsed with PBS and 100 µl of an AB/NR medium (5% [v/v] solution of AB and 1.25% [v/v] of NR dye) prepared in fresh media (without FBS or supplements) were added to each well. Following 3 h incubation, AB fluorescence was quantified at the respective excitation and emission wavelength of 540 nm and 595 nm. Wells containing medium and AB without cells were used as blanks. The mean fluorescent units for the six replicate cultures were calculated for each exposure treatment and the mean blank value was subtracted from these. Following the AB assay, the NR dye was extracted from the cells using a ethanol:water: acetic acid solution (50:49:1%). NR fluorescence was then quantified at the respective excitation and emission wavelength of 595 nm and 650 nm.

The percentage of growth inhibition (GI%, or % cytotoxicity) was calculated according to the following formula [32]:

\[
\%GI = 100 - 100 \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}}
\]

where \(A_{\text{sample}}\) is the absorption value of the test extract; \(A_{\text{blank}}\) the absorption value of the blank cultures (without cells); \(A_{\text{control}}\) is the absorption value of the solvent control.

6.2.4 Silver leaching from Ag nano-particles combined with polymer

Ag and Ag\(^+\) leaching out from silver nano-particles combined with polymer were studied by Ag-polymer coated on first-aid bandages and immersed in water. The concentration of silver leaching out from the coatings was measured by AAS (Varian, Spectra AA200) as a function of time.
6.3 Results and Discussion

6.3.1 Preparation and characterisation of silver nanoparticle-PVA composites

The details of preparation of silver nano-particles were described in Chapter 5 experimental part. The solution after dilution in ethanol was characterized by UV-vis spectroscopy and the results were shown in Figure 6.1. The peak at ca. 390-400 nm showed the absorption spectra of silver nano-particles at room temperature and exposed to air. Silver nano-particles were still stable after crosslinking PVA with adipic acid under proper conditions.

![UV-vis spectra for the samples containing colloidal Ag nano-particles: Ag-PVA prepared after 2 hours (AgNP-PVA) and Ag-PVA crosslinked with adipic acid (AgNP-PVA-CL)](image)

The samples employed to test antibacterial activity were characterized by XRD to conform the existence of silver nano-particles stabilized in PVA. The result was shown in Figure 6.2. The peaks sitting at 38.5 degrees, 44.5 degrees, and 65.5
degrees showed (111), (200), and (220) diffraction of face centered cubic (fcc) silver crystals [33,34].

Figure 6.2 XRD pattern from the film of the silver nano-particles stabilized with PVA dried in a microslide

Figure 6.3 TEM image of silver nanoparticle stabilized in PVA crosslinking with adipic acid
Chapter 6 The Antibacterial Activity of Silver Nano-particles Stabilized with PVA

The morphology of silver nano-particles stabilized with PVA crosslinked with adipic acid was investigated by TEM. Figure 6.3 shows the TEM image of silver nano-particles stabilized with PVA crosslinked by adipic acid after dilution in ethanol at room temperature. The silver nano-particles were cast in the PVA polymer film and also there are some pores left in the polymer film by the evaporating solvent.

6.3.2 Antibacterial activity testing

The antibacterial activity of silver nano-particles stabilized with PVA and silver nano-particles stabilized with PVA crosslinked by adipic acid for *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) was measured by an international recognised standard procedure. The blank tests of solvent of ethanol, the effect of pH values were also investigated for measuring the antibacterial activity of silver nanoparticle composites. The silver nano-particles stabilized with PVA crosslinked by adipic acid showed high antibacterial activity to both *E. coli* and *S. aureus* (Figure 6.4) while silver nano-particles stabilized with PVA showed slight antibacterial activity. The growth inhibition rings of *E. coli* and *S. aureus* treated with silver nano-particles stabilized with PVA crosslinked by adipic acid were 3cm and 3.4cm. This may be caused by the dissolution of silver nano-particles to silver ions more easily by the crosslinking of PVA in adipic acid than those stabilized with PVA only. Ethanol showed very weak antibacterial activity and adipic acid displayed almost no antibacterial activity. However, the pH value of the solution did affect the antibacterial activity. The pH value of silver nanoparticle crosslinked with PVA in adipic acid was slightly higher than 2 at
which pH value a solution without silver nanoparticles did not show any antibacterial activity.

The MRD (Maximum Recovery Dilution) method was employed to investigate the growth inhibition effect of silver nano-particles stabilized with PVA crosslinked by adipic acid. Figure 6.5 showed the results of the capability of silver nano-particles stabilized with PVA crosslinked by adipic acid for its antibacterial activity by diluting the concentrations to half of previous one. The clear solution in the specimen bottles indicated that the bacteria did not grow; in another words, the bacteria were either killed or inhibited from growth in the medium containing silver nano-particles. The creamy colour of the solution showed the growth of the bacterial in the medium which lost its antibacterial activity. When the concentration of silver nanoparticles in the medium decreased to ca. 1ppm, it lost

Figure 6.4 The results from antibacterial susceptibility testing (AST) on the silver nano-particles stabilized with PVA (crosslinked): left, *E. coli*; right, *S. aureus*;

The MRD (Maximum Recovery Dilution) method was employed to investigate the growth inhibition effect of silver nano-particles stabilized with PVA crosslinked by adipic acid. Figure 6.5 showed the results of the capability of silver nano-particles stabilized with PVA crosslinked by adipic acid for its antibacterial activity by diluting the concentrations to half of previous one. The clear solution in the specimen bottles indicated that the bacteria did not grow; in another words, the bacteria were either killed or inhibited from growth in the medium containing silver nano-particles. The creamy colour of the solution showed the growth of the bacterial in the medium which lost its antibacterial activity. When the concentration of silver nanoparticles in the medium decreased to ca. 1ppm, it lost
its antibacterial activity for *E. coli*. When the concentration of silver nanoparticles was diluted to 0.2 ppm, it still had antibacterial activity for *S. aureus*. This indicated that *S. aureus* was more sensitive to silver nanoparticles than *E.coli*.

![Figure 6.5 The MRD results of silver nanoparticles stabilized in PVA crosslinked by adipic acid on E. coli inhibition growth test](image)

Specimens were taken from those samples which had antibacterial activities for *E. coli* and *S. aureus* and were cultured under the conditions suitable for bacteria growing. It was found neither *E.coli* nor *S. aureus* in the specimens grew. This suggested that silver nano-particles killed both *E. coli* and *S. aureus*.

Cho et al. [35] studied the antibacterial activity of nanosilver stabilized by poly-(N-vinyl-2-pyrrolidone) (PVP) for *E. coli* and *S. aureus*. Cho’s results showed that silver nano particles stabilized with PVP in 50ppm completely inhibited the growth of *S. aureus* while in 100ppm for inhibiting the growth of *E. coli*. Cho also suggested that silver nano particles had antibacterial activity against *E. coli* and *S. aureus* by disrupting the surface of the cell walls. Sondi *et al* [36] investigated the behaviour of silver nanoparticles prepared from silver hydrosol against *E. coli*.
They found that even at high concentrations, silver nanoparticles in liquid medium only delay the growth of *E. coli* for a few hours because of low colloidal stability of silver nanoparticles in liquid medium. They also observed the damage to membranes of dead *E. coli* cells. Silver nanowire/mesoporous silicate nanocomposites have been synthesized by Zhang *et al.* [3] and their samples exhibit a highly inhibitory effect on the growth of *Escherichia coli* (ATCC 25922). Silver nano-assembly was synthesized based on conventional Tollen’s reaction by Sarkar *et al.* and their silver nano particle in solution displayed very high anti-microbial activity [37]. Their results showed an evident in very low inhibitory concentration value of 4 ppm for *Escherichia coli*, *Vibrio cholerae*, *Shigella flexneri*, *Salmonella typhimurium* and three varieties of *Staphylococcus aureus*. They suggested that the low MIC value was comparable to that of Penicillin in the cases of *E. coli* and *S. aureus*. The silver nanoparticles polymeric composite prepared in this work showed even lower concentrations against *E. Coli* and *S. aureus*. The concentration for *E. Coli* was as low as 2 ppm and 0.2 ppm for *S. aureus*.

### 6.3.3 In vitro cytotoxicity test

Cytotoxicity of silver nanoparticle stabilized with PVA crosslinked in adipic acid to A549 cells following 24 h exposure was quantified by the Alamar Blue (AB) and the Neutral Red (NR) assays. The Alamar Blue assay was used to assess metabolic activity and the Neutral Red assay was used for the assessment of membrane function and lysosomal activity[22,23,30,31].
Figure 6.6 showed the tests of cytotoxicity of silver nanoparticle stabilized with PVA crosslinked with adipic acid (Ag-NP-PVA-CL) to A549 cells after 24 h exposure determined by the AB assay and NR assay. Figure 6.7 showed the cytotoxicity of samples of Ag-NP-PVA-CL after being filtrated or autoclaved to A549 cells under same test conditions. When the concentration of the samples was low, it did not show cytotoxicity. With increasing the concentration up to 20ppm, there was no significant cytotoxicity that is defined as an effect leading to an inhibition of cell growth (GI%) more than 30% as compared to the cultures treated with negative controls [8]. The sample after autoclaved showed much more cytotoxicity compared the sample without any further treatment while the filtered sample showed less cytotoxicity.
Yu et al. [8] prepared the silver nanoparticles stabilized with poly glutamic acid (PGA/Ag0) by dextrose reduction method. The in vitro cytotoxicity was evaluated by L929 fibroblasts proliferation and the antibacterial activity against methicillin-resistant *S. aureus* (MRSA) and *P. aeruginosa* was assessed. Their results showed the PGA/Ag0 displayed no evidence of causing cell lysis or toxicity. They also found that the cyto-compatibility of PGA/Ag0 was better than PGA/Ag+.

### 6.3.4 Silver leaching from Ag nano-particles combined with polymer

Ag and Ag+ leaching out from silver nano-particles combined with polymer were studied by Ag-polymer coatings on first-aid bandages and immersed in water. The concentration of silver leaching out from the coatings was measured by AAS. The results are shown in Figure 6.8. Silver leached from the first-aid bandage into
water quickly at the beginning in a diffusion control process and then went to a steady state. The highest concentration was 3.5ppm in 25ml water.

![Graph](image)

**Figure 6.8** silver leaching from Ag nano particles combined with polymer (PVA) coated on first-aid bandage measured by AAS (0.2ml nanosilver composite in 25ml water)
6.4 Conclusions

Silver nano-particles stabilized with PVA crosslinked with adipic acid showed high antibacterial activities to both *E.coli* and *S. aureus* while silver nano-particles stabilized with PVA showed slight antibacterial activity. The pH value affects the antibacterial activity of silver nanoparticle composite.

Preliminary cytotoxicity test of silver nano particles stabilized with PVA crosslinked with adipic acid to A549 cells showed that the sample did not have significant cytotoxicity when the concentration increased up to 20ppm.
6.5 References


Chapter 6 The Antibacterial Activity of Silver Nano-particles Stabilized with PVA


Chapter 7 Conclusions
Silver is capable of killing bacteria and pathogens and therefore has found use as a biocidal agent, often being incorporated into biomedical devices. These include urinary tract catheters and wound dressings [1]. Most research work performed in the past on silver oxidation or dissolution related to fundamental electrode processes. This work focused on silver dissolution behaviour from pure silver and silver-platinum alloys, as well in combination with polymeric materials under the conditions suitable for applications of medical devices and hygienic coatings.

Silver dissolution behaviour from pure silver and silver-platinum alloys in solutions both in the presence and absence of chloride was investigated using cyclic voltammetry (CV). In the absence of chloride, silver dissolved to form silver ions in an activation controlled process and diffused away from the surface of the sample. In the presence of chloride, silver chloride was firstly formed in a diffusion controlled process. This process dominated the dissolution behaviour of silver from both pure silver and silver-platinum alloys.

For a series silver-platinum alloys (up to 5%wt of platinum) studied, it was found that there was no significant difference for silver dissolution behaviour or surface morphology characterized by SEM and EDX. However, the nucleation of AgCl formed in the oxidation process and the nucleation of Ag formed in the reduction process occurred for all samples but the addition of Pt inhibited the nucleation of AgCl and the formation of Ag$^+$ on repeated CV cycling. The AgCl grew on top of the sample and the reduced Ag formed a porous layer on top of Ag-Pt alloy. The layer of reduced Ag formed on the Ag-Pt samples had a larger pore size than that formed on pure silver. The degree to which silver is oxidized, decreased with increasing platinum content both in the absence and in the presence of chloride. The value of the silver dissolution potential on the fresh surface of each sample
and the value of the zero current potential increased with increased level of Pt in the alloy.

A Zero Resistance Amperometry (ZRA) measurement was carried out to further study the effect of platinum on the silver dissolution behaviour in 0.89%wt. NaCl solution. ZRA results for a two electrode cell showed that platinum did enhance silver dissolution through a galvanic reaction by increasing the area of the platinum electrode.

The mechanism of silver dissolution from pure silver and silver-platinum alloys in 0.89%wt. NaCl solution was modelled by an equivalent circuit model using electrochemical impedance spectroscopy (EIS). The model consisted a double layer formed on the rough and porous surface of silver or silver-platinum samples; a resistance produced by charge transfer through the double layer; a layer of silver chloride formed by the diffused silver ions and the chloride ions from solution, which produced a resistance as well. The difference of silver dissolution behaviour between silver and silver-platinum alloys is that the resistance associated with the electron transfer of the silver-platinum alloy is much higher than that of pure silver. This result supports that of the CV study.

Silver and silver-platinum alloy coatings on polymeric materials of PA, PE, PVC and silicone were deposited using an atom beam and magnetron sputter coating system. However, the adherence of the silver plasma coating was very poor on the surfaces of the polymers. Silver dissolution behaviour from the plasma coatings on the polymeric material was investigated by cyclic voltammetric technique in 0.89%wt. NaCl solution. The dissolution behaviour of silver from the plasma coatings on polymers displayed similar behaviour to those from pure silver or
silver-platinum alloys. AgCl formed when silver was oxidized in the solution containing chloride and the film lifted away from the polymers.

The morphology of the thin metal coatings depends on the type of polymer as well. The coating on silicone sheets showed the metal existing as irregular particles while uniform films formed on the other polymers. This could be a factor affecting the silver dissolution behaviour from the plasma coatings on the polymeric materials.

Novel polymeric composites containing silver nanoparticles were prepared by the reduction of silver nitrate with sodium borohydride under the protection of capping polymers of PVA, PVC, PVP or PEG. The properties of the silver nanoparticle polymeric composites were characterized by UV-vis spectroscopy, XRD, TEM, SEM and EDX, as well as Raman spectroscopy. The stabilities and morphology is dependent on the properties of the polymers: PVA and PVC showed very high stability to the silver nanoparticles, while silver nano-particles formed with PVP and PEG were unstable. The size and the distribution in size of silver nano-particles were also dependent on the type of polymers. This was confirmed by results of UV vis spectra, XRD patterns and TEM images of silver nano-particles. PVC as a capping agent is a novelty in this work and it allows the possibility of the nanoparticles being formed in non-aqueous solvents. This would allow films to be cast more quickly.

It was seen for PVA that the Raman scattering for the C-O stretching and O-H bending bonds was enhanced due to the presence of Ag nanoparticles.

The silver dissolution behaviour from the polymeric silver nanoparticle composite was investigated by cyclic voltammetry in 0.89%wt. NaCl solution and had a similar behaviour to that of pure silver or the samples of polymer plasma coated
with silver. The advantage of the silver nanoparticle polymeric composite is the reproducibility of oxidation and reduction CV peaks for the silver/silver chloride couple and the stability of the silver nanoparticle polymeric composite compared to plasma coated polymeric material.

The antibacterial activities against strains of *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) of silver nano-particles polymeric composite were investigated. Silver nano-particles stabilized with PVA crosslinked with adipic acid showed high antibacterial activities to both *E. coli* and *S. aureus* while silver nano-particles stabilized with PVA showed slight antibacterial activity.

The Alamar Blue (AB) assay and the Neutral Red (NR) assay were employed to assess metabolic activity, the membrane function and lysosomal activity of A549 cells to evaluate the cytotoxicity of the polymeric silver nanoparticle composite. Preliminary cytotoxicity tests of silver nano particles stabilized with PVA crosslinked with adipic acid to A549 cells showed that the sample did not have significant cytotoxicity when the concentration increased up to 20ppm, at which concentration it showed strong antibacterial activity.

In conclusion, this work examines whether the inclusion of Pt is of benefit to silver dissolution or not. This process depends on the galvanic reaction which takes place between silver and platinum. In fact for cyclic voltammetry the rate of silver oxidation decreases with increasing platinum in the presence of chloride, perhaps due to platinum disrupting the nucleation process for silver chloride. This is confirmed by EIS. The development and characterisation of silver composites show that the novel silver nanoparticle polymeric composite which was crosslinked with PVA, PVP and 1,6 Dibromohexane is not water soluble and more rugged than sputter coatings for Ag⁺ delivery. These novel films can be cast
on materials with complex geometries, such as catheters, unlike those from sputter coatings. These can deliver silver ions in a diffusion controlled manner as shown by AAS due to high available surface area of silver. The silver nanoparticles in PVA crosslinked with adipic acid have been shown to be effective as an antibacterial agent against *E. coli* and *S. aureus* without causing cytotoxicity.

Future work should include the following:

- Optimise the ratio of the crosslinking agents, such as adipic acid to PVA.
- Employ these films with catheters.
- Determine if Atomic adsorption spectroscopy can distinguish between Ag and Ag$^+$
- Further examination of PVC as a capping agent in THF with a view to forming novel composites for use in films.
- Examine alternative uses for these silver nanoparticle polymeric composites, for example, gas sensing.
- Determine the potential profile of the silver platinum alloy by AFM to assess whether there is the possibility of local corrosion.

Reference: