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Determination of silver release from wound care products

Master of Science Thesis in Materials Chemistry and Nanotechnology

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Abstract

Silver is used as an antimicrobial agent in wound care products due to its effectiveness against a broad range of bacteria. Despite extensive use of silver-containing dressings, silver release measurements vary significantly for instance in release method and test fluid used, thus making it hard to compare results. In addition, existing methods for silver release involve the dressing being totally saturated with test fluid, this however does not mirror the real situation in wound treatment. The aim with this master thesis is to develop a test method for measuring silver release from silver-containing dressings that are not fully saturated with test fluid. Silver release at different degrees of moisture saturation was investigated using inductively coupled plasma optical emission spectroscopy.

In order to simulate wound-like conditions pieces of dressings were pre-wetted with test fluid to a certain moisture level and placed on an acceptor compartment (AC) containing a defined amount of salts and proteins. After incubation, the AC was digested in acid prior to analysis with ICP-OES.

Results show that silver release depends on the test fluid used. For deionized water a silver release of at most 19 μg Ag/cm^2 AC was obtained. For solution A, which consists of distilled water with NaCl and $CaCl_2$, a release of 1 μg Ag/cm^2 AC was found. Silver release for SWF, which consists of fetal calf serum mixed with equal amount of peptone water, was 4 μg Ag/cm^2 AC. Measurements performed on 0%, 25%, 50%, 75% and 100% moisture saturation show that silver release is dependent on the moisture saturation of dressing. A higher silver release was seen for dressings pre-wetted to higher moisture saturations. Digestion of the acceptor compartment prior to analysis with ICP-OES was found to be satisfactory. Finally, silver release on human skin was performed in order to evaluate the relevance of the method being developed. Results show good agreement between silver release in the acceptor compartment model used and skin as acceptor compartment which strengthens the relevance of the developed method.

Keywords: silver, release, dressing, wound, ICP.

Abbreviations

AAS	Atomic Absorption Spectroscopy
AC	Acceptor Compartment
ATR	Attenuated Total Reflectance
FCS	Fetal Calf Serum
FTIR	Fourier Transform Infrared Spectroscopy
ICP-MS	Inductively Coupled Plasma Mass Spectroscopy
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
Mölnlycke	Mölnlycke Health Care
POM	Polyoxymethylene
SEM	Scanning Electron Microscopy
SWF	Simulated Wound Fluid

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1. Introduction

The normal amounts of microorganisms in humans are estimated to be 10^{14}, most of which are not pathogenic. Still, some of these microorganisms can cause infections. All chronic wounds are colonized with bacteria which delays wound healing (Lindholm, 2010). Hard to heal wounds like venous leg ulcers and pressure ulcers are a great concern since they cause pain and discomfort to patients and increases treatment costs for the health care sector.

The use of silver as an antimicrobial agent has been recognized since ancient times. Settlers who came to western America placed silver dollars in their water barrels to preserve the water. In 1884 a 1% silver nitrate solution was used to prevent eye infections in infants (Burell, 2003). However, the incorporation of silver in wound dressings is a relatively new invention and was first investigated in 1987 (Thomas, 2010). Silver is used in dressings because of its antibacterial effect against a broad range of aerobic, anaerobic, Gram-positive, Gram-negative bacteria, fungi, viruses and yeasts (Burell, 2003). Nowadays there are a large number of silver-containing dressings available on the market, claiming to be effective against a wide range of bacteria over multiple days. The dressings differ significantly regarding the silver content and substance, silver release kinetics and material properties.

Studies have shown that silver release from different silver-containing dressings is widely affected by the silver release methods and the test fluid used (Walker et al., 2006; Rigo et al. 2012; Lindsay et al. 2010). In addition, all silver release methods available involve that the dressings are totally saturated with test fluid. This does not mirror wound like conditions and does not make it possible to measure silver release at different moisture saturations. A system that simulates the wound environment to a better extent is therefore necessary in order to obtain credible results of silver release. A better understanding of silver release from silver-containing products may lead to development of new and improved dressings.

To be able to measure silver release at different moisture saturations it is relevant that the dressing is absorbent. The test methods used in this project is therefore developed for absorbing wound dressings and materials.

1.1 Aim

This master thesis aims to develop a method to measure silver release from silver-containing dressings and materials at different levels of moisture saturation. A semi-solid matrix that mimics the conditions in the wound is required as acceptor compartment. The silver concentration is determined by inductively coupled plasma optical emission spectroscopy (ICP-OES).

2. Background

The wound healing process and use of silver in wound care products will be explained in this chapter. Also, different methods for dissolving silver from wound care dressings will be presented together with analytical techniques for measuring silver as well as sample preparations before analysis.

2.1 Silver in wound care

The skin is the largest organ in the body and has several important functions like regulation of body temperature, protection from external threats and storage of body fluids to mention a few. The skin is divided into two main parts, *epidermis* which is the superficial and thinnest part and provides a protective barrier and *dermis* which is the deeper layer which provides flexibility and strength to the skin. Wound healing is a complex process where the skin repairs itself after an injury.

Wounds can be divided into two groups; acute wounds and chronic wounds, the latter often called hard to heal wounds. Acute wounds usually include surgical wounds, traumatic wounds and burns. These wounds occur suddenly and normally heal after a shorter time. Chronic wounds are usually caused by a disease or tissue damage and the healing time is often long (Lindholm, 2003). The normal wound healing process consists of three phases; inflammatory phase, proliferative phase and remodeling phase (Lindholm, 2003). The inflammatory phase lasts for approximately 3-4 days but for chronic wounds inflammation in the wound is active during the entire healing time. During this time blood vessels expands and white blood cells accumulate around the injury to protect the wound against infection by killing bacteria (Lindholm, 2003). The proliferative phase is characterized by regeneration of tissue that has been lost or damaged. This stage lasts for 3-4 weeks for acute wounds. The final stage in the wound healing process, the remodeling phase, can last up to several years and includes processes to increase the flexibility in the tissue.

Bacteria are considered to be one of the main reasons for why wound healing may stall, and colonized and infected wounds may lead to discomfort for the patient, possibility of life-threatening illness and delayed wound healing. Silver has been recognized for its antimicrobial effect since ancient times, long before the existence of microorganisms were first suspected. Due to its effectiveness against a broad range of bacteria silver is nowadays used as an antimicrobial agent in wound dressings. Numerous silver-containing dressings are available on the market with different properties regarding material, release kinetics, amount of silver and silver substance. It is

however shown that there is no correlation between silver release and total content in the dressing (Hamberg, 2012).

Silver is widely used in medical applications and is employed in catheters, dentistry, prostheses, surgical needles and water purification to mention a few (Lansdown, 2004). Silver is a precious metal, is less reactive compared to other elements and does not react with human tissue in its non-ionized form. However, when in contact with moisture, wound fluids and exudates silver readily forms Ag^{+} which is generally accepted to possess the antimicrobial effect. Silver may also form Ag^{2+} and Ag^{3+}, but these are unstable and therefore rare (Lansdown, 2004). In order for silver release from dressings to occur, different silver salts and complexes are used as carriers which are dissolved when in contact with fluids, thus releasing Ag^{+}. According to Lansdown and Williams (2004) silver is incorporated into wound dressings as:

- Elemental silver – nanocrystalline particles or foil
- Inorganic compounds/complexes – silver sulphate, silver nitrate, silver sulphadiazine, silver oxide, silver phosphate, silver chloride or a silver zirconium compound
- Organic complexes – colloidal silver preparations, silver-zinc allantoinate or silver proteins.

Silver can be distributed in different ways within the dressing. Some dressings have silver evenly distributed in the entire material while other dressings only have silver present on the wound contact surface. Some dressings consist of an absorbent material while others cannot absorb moisture.

Silver ions bind readily to thiol-groups on proteins (including albumins and metallothioneins) and interact with trace metals in metabolic pathways (Thomas, 2010). According to Lansdown (2004) silver ions absorbed by sensitive strains:

- Impairs bacterial cell wall integrity
- Binds and disrupts subcellular components
- Inhibits respiration
- Impairs essential enzymes and metabolic events modulated by sodium, magnesium, phosphate, etc.
- Inactivates bacterial DNA and RNA.

Previous studies have shown that silver release from wound dressings is greatly dependent on the type of medium (Walker et al. 2006, Rigo et al. 2012 and Lindsay et al. 2010). Body fluids contain proteins and salts to which silver ions can readily bind and form precipitates. Wound

exudates contain a high percentage of chloride ions (Cl^-) that Ag^+ can bind to. This will lead to formation of AgCl which has a low solubility ($K_{sp} = 1.77*10^{-10}$ at 25°C) (Lide, 1990). The common opinion is that silver chloride does not possess an antimicrobial effect, resulting in reduced antimicrobial effect when silver ions come in contact with chloride ions in wound exudates. The silver ion most likely also binds to other compounds in exudates. However, the amount of silver that has to be present in the wound for antimicrobial effect is debated. Some argue that a high amount of silver release is better, while others believe that a slow silver release is to prefer. Still, there is no information on what silver concentration that should be present in the wound since no biopsies have been performed. However, Burrell (2003) has reported that as low as 0.01 µg/mL silver in an aqueous system may be sufficient to control bacteria. Nevertheless, a higher silver content is probably required in wounds.

The increase in exposure of silver has raised the concern about possible resistance. Silver is not solely used in medical applications but also incorporated into commercial products like shoes, socks and underwear due to its antibacterial properties and prevention of odor. This extensive use increases the risk of silver resistance, however the mechanism behind it is poorly understood. Some argue that since silver does not target a specific site on the cell but attacks multiple sites the risk of resistance to silver is reduced (Percival et al., 2005). The fact that resistance to antibiotics is a large concern is that antibiotics target a specific site, thus increasing the possibility of resistance. Even if the probability of resistance to silver is reduced due to the multifunctional properties studies have shown silver resistance in some bacteria. Therefore, silver resistance cannot be ignored and more research has to be made in that area.

2.2 Silver release measurements from silver-containing dressings

Silver release has become a well studied property for silver-containing dressings, both for predicting antimicrobial effect and for safety reasons. There is currently no standard method available for silver release from dressings. Therefore, wound dressing manufacturers develop their own methods. This results in a number of methods available for dissolving/releasing silver ions from wound care dressings into solutions. For the methods described below a number of important parameters can be varied, for example test fluids used, rotation/shake, size of dressing, temperature of incubation, covering of the system, time intervals for silver release measurements, volume of test fluid and detection method.

Beaker method / shake flask

The beaker method is a simple method for measuring silver release from products that are fully saturated with test fluid. The method is widely used for silver release measurements (Cavanaugh et al, 2010; Wright et al. 1998; Lindsay et al. 2010; Parson et al. 2005 and Walker et al. 2006) as it is easy to perform and requires simple equipment. A piece of dressing or material is added to a beaker with a defined amount of test fluid. For silver release measurements, samples are taken out at defined time intervals. The advantage with this method is its simplicity, however it does not mirror the real situation in wound environment since the product or material is fully saturated and exposed to an excess of test fluid. The disadvantages with this method are that the products may stick to the beaker, products may float and because of this the method does not take into account the different sides of the product.

Diffusion cell

Diffusion cell is a method used for studying transfer kinetics through membranes (Hanson, 1991). One type of diffusion cell is Franz Cell diffusion, see Figure 1. This method is used for pharmaceutical formulation, mainly for topical and transdermal drug delivery formulations. Franz Cell diffusion consists of two compartments separated by a membrane. The donor and acceptor compartment are held together using a clamp. The acceptor compartment is filled with test fluid. Samples are withdrawn at specific time intervals through the sampling port and volume is replaced in the same manner. The method can be modified for wound dressings (Schwarzkopf et al. 2010, Dolmer et al. 2004). Then the dressing serves as the donor compartment. The advantage with the Franz Cell is that a water jacket is present, thus giving a temperature control of the acceptor compartment.

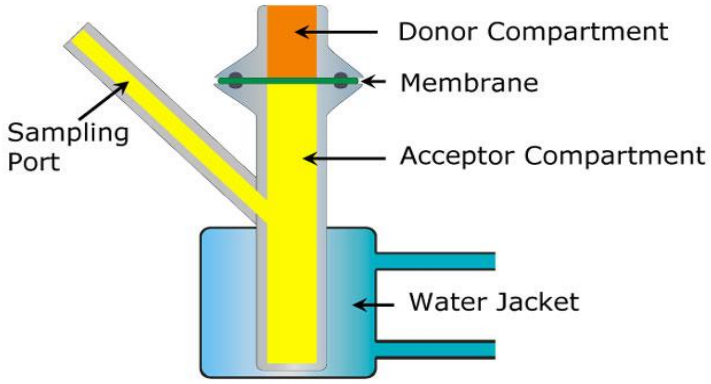


Figure 1 Schematic illustration of Franz diffusion cell (The European Virtual Institute for Specification Analysis, 2010).

Paddle over disc

The paddle over disc method is an official technique for in vitro testing of transdermal devices (Hanson, 1991). This method consists of a rotating paddle with a blade and a disc that holds the transdermal product at the bottom of the flask, with the wound contact surface towards and parallel with the paddle blade (Hanson, 1991; US Pharmacopeia, 2006). For a schematic illustration of the method, see Figure 2. The flask is placed in a water bath so that the temperature of the system can be controlled. Test fluid is added to the flask. The paddle provides agitation of the test fluid and test fluid specimens are collected for evaluation at given time intervals. However, since the product is fully saturated with test fluid as well as exposed to an excess of test fluid it does not mirror the real situation in wound environment. The method can be modified for wound dressings with silver, but no references to confirm this was found during this thesis work.

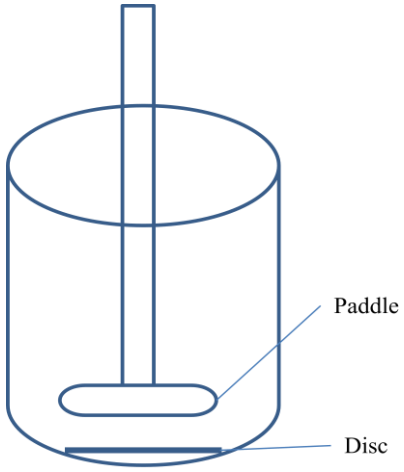


Figure 2 Schematic illustration of Paddle over disc.

Two compartment model

The two compartment model was developed at Mölnlycke (Hamberg et al., 2012) in order to measure silver release in products where only one side of the product is in contact with the test fluid, see Figure 3. The two compartment model uses 6-well plates with lids together with inserts in which pieces of products are placed. Test fluid is added to the wells and thereafter the inserts are placed in the wells. The inserts can have membrane or be modified so that the membrane is replaced with a plastic film with one big hole. Also, a weight may be placed on top of the product. The aim with this method is to improve the simulation of wound environment to a better extent than the previous presented methods.

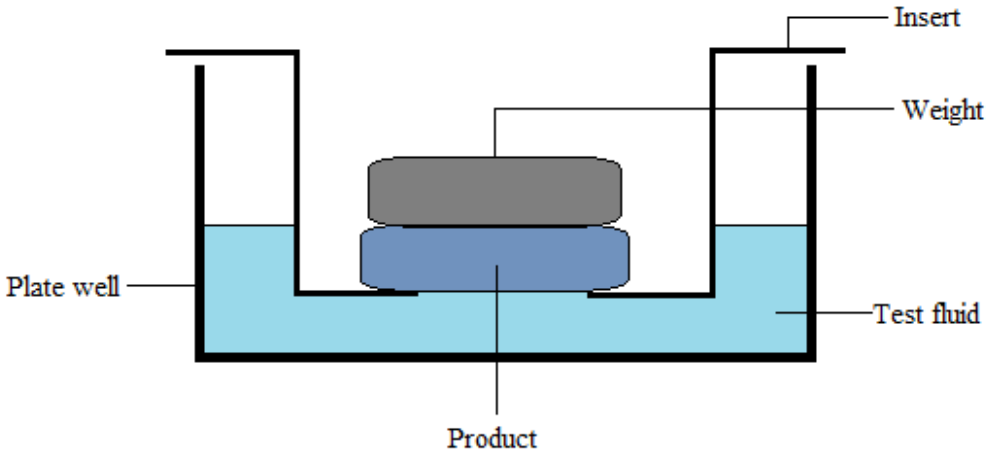


Figure 3 Schematic illustration of two-compartment model.

The previously described release methods involve that the dressings are fully saturated with test fluid. This may not mirror the usage of silver-containing dressings in healthcare at which the dressing is not totally saturated during use. Development of a new method for silver release measurements from dressings that are not fully saturated is needed to obtain a system that simulates a wound like environment.

2.3 Analysis of silver

The methods for silver release presented in chapter 2.2 are used to extract silver from the dressings. Methods for analyzing the silver content are subsequently necessary in order to determine silver release. A number of common techniques for silver determination are presented in this section. Finally, to be able to measure silver in the above mentioned methods different sample preparation methods including digestion are needed.

2.3.1 Sample preparation

Sample preparation involves that the sample is transformed to make it suitable for analysis. This may include dissolving the sample, extracting analyte from matrix, concentrating an analyte so it becomes measurable, chemically convert the analyte into a detectable form, and removing or masking interfering species (Harris, 2007). When choosing a suitable method for sample preparation several factors have to be considered as the type of analytical method for metal analysis, type of matrix in which the element to be measured exist and the concentration range of the element (Kebbekus, 2003). The most common sample preparation methods involve digestion with acid and dry ashing. Worth mentioning is that these methods will not give any information about the original amount of silver ions released from the dressing.

Acid digestion

Acid digestion is the most common type of sample preparation method for elemental analysis such as ICP-MS, ICP-OES and AAS. Nitric acid is the most frequently used acid since it prevents formation of insoluble silver salts (Kebbekus, 2003).

The matrix with the element to be analyzed is weighed and placed in a beaker together with the chosen acid(s). The beaker is covered and heated. More acid can be added to prevent the matrix from drying out (Kebbekus, 2003). When the matrix is fully dissolved it is evaporated and then taken up in a dilute acid solution and diluted to volume for analysis (Kebbekus, 2003). Several factors have to be considered when choosing the appropriate acid for digestion. If possible the mildest acid that will dissolve the matrix with the element of interest should be used, thus providing a safer environment in the laboratory and minimizing the risks of acid attack on tubes when measuring silver analysis with ICP for example. Hydrochloric acid together with nitric acid can be used to improve the digestion of the matrix. Sulfuric acid may be used if further digestion is necessary. For digestion of matrices with noble metals like silver and gold, aqua regia (3:1 volume ratio mixture of concentrated hydrochloric and nitric acid) may be used (Kebbekus, 2003), see reaction 1 and reaction 2 in Figure 13. Finally, sulfuric acid with hydrogen peroxide and hydrofluoric acid can dissolve all metals and alloys, as well as minerals, soils, rocks and sediments (Kebbekus, 2003).

Microwave digestion

Microwave digestion is a wet-ashing procedure that includes digestion in microwave with acid in a Teflon bomb (Harris, 2007). It is a common technique used for digestion of metals in organic matrices. Digestion is performed in a closed vessel made of high-temperature polymers like PTFE. The matrix to be digested is placed inside the vented vessel and exposed to acid. Both pressure and temperature inside the vessels can be controlled. Digestion is often divided into stages where the temperature and pressure are slowly raised. In addition, the digestion time as well as oven power can be programmed so that each sample is subjected to the same conditions. Microwave digestion has been successfully used for determination of trace levels of mercury in foliage (Rea et al. 1998) using nitric acid. The advantages with microwave digestion compared to open container dissolution methods is the smaller risk of metal contamination from the high-temperature polymer vessels compared to beakers or crucibles (Kebbekus, 2003). Also, since digestion is performed in closed vessels the risk of evaporation of volatile metals is reduced and less acid solution for digestion is required.

Ultrasound-assisted extraction

Ultrasound assisted extraction uses sound waves to create mechanical vibrations in solids, liquids and gases (Priego-Capote, 2004). Sound waves together with extremely high temperatures and pressure cause implosions in the liquid and lead to extraction of the sample. Ultrasound-assisted extraction has in recent years emerged as an efficient method for sample preparation for trace element analysis using both probe and bath ultrasonic processors and has been used as a sample pre-treatment when analyzing arsenic, selenium, nickel and vanadium in fish and shellfish by electrothermal atomic absorption spectrometry (Lavilla, 2007). Digestion is carried out by placing the sample in a flask with extraction solvent. The flask is immersed in an ultrasound bath and by controlling the amplitude of sonication, time of extraction, solvent ratio, temperature and frequency good extraction can be achieved (Priego-Capote, 2004). The advantages with ultrasound-assisted extraction compared to conventional digestion methods like acid digestion include simplicity of use, no need of expensive equipment and no risk of evaporation during digestion. It is often used with the sole aim of saving time (Priego-Capote, 2004).

Dry ashing

Dry ashing can sometimes be used to facilitate sample preparation. It can be used if the compound to be analyzed is present in extremely low concentrations or if digestion methods normally used is not enough. Also, some samples are very difficult to digest, and by oxidizing the sample it will be easier to dissolve. Dry ashing is a relatively simple method for analysis of non-volatile metals in organic matrices and can be used for measuring nutritional elements in food like iron, potassium, calcium and magnesium among others (Kebbekus, 2003). The matrix is placed in a crucible which is put in a muffle furnace. The temperature in the furnace is an important factor since too high temperatures can result in volatilization of the element to be analyzed. A temperature of approximately 400 to 450°C is recommended. Salts and sulfuric acid may aid in the dry ashing process since salts helps retain some elements thus avoiding volatilization and since sulfuric acid has a chemical charring effect (Kebbekus, 2003). However, difficulties may arise with fats and oils since these compounds ignite, thus leading to possible loss of the element of interest. The combusted ash is dissolved with concentrated nitric acid and water.

2.3.2 Methods for silver analysis

There are a number of quantitative methods available for detection of silver. The methods presented below measure the amount of silver or silver ions in a fluid, using calibration standards except for Volhard titration method.

Silver ion electrode

Silver ion electrode is a simple and quick method for measuring silver ion concentration by placing the silver ion electrode in a water solution. The silver ion electrode consists of a thin membrane that only attracts and detects the ion of interest (Harris, 2007). The membrane consists of a ligand that has a high affinity for the ion of interest. Hence, the method is not suitable for measuring silver ions in protein solutions or solutions with interfering ions like chloride. In addition, the method only measures silver ions (Ag^+), and not elemental Ag. Unwanted ions can also attach to the ligand which may interfere with the result from the electrode.

Titration method (Volhard method)

The Volhard method is an end-point detection method for determination of Ag^+ that form complexes with Cl^-, Br^- and I^-. Titration of Ag^+ is performed in HNO_3 solution and for Cl^-, a back titration is necessary (Harris, 2007). Cl^- is precipitated by excess of $AgNO_3$ standard.

$$Ag^+ + Cl^- \rightarrow AgCl\,(s)$$

AgCl is filtered and washed, and the excess Ag^+ in the filtrate is titrated with potassium thiocyanate (KSCN) solution together with Fe^{3+} (Harris, 2007).

$$Ag^+ + SCN^- \rightarrow AgSCN\,(s)$$

In this reaction silver ions react with thiocyanate ions and form silver thiocyanate precipitate. When all silver ions have reacted, the slightest excess of thiocyanate will react with Fe^{3+} to form $FeSCN^{2+}$ which is a red complex (Harris, 2007).

$$Fe^{3+} + SCN^- \rightarrow FeSCN^{2+}$$

When the solution changes color the titration is finished and the volume of KSCN is recorded. The available amount of silver ions in the solution can be calculated with this method. In a solution where it is assumed that only silver ions forms complexes with thiocyanate, the concentration of Ag^+ can be determined.

Atomic absorption spectroscopy (AAS)

Atomic absorption spectroscopy (AAS) is a spectroanalytical method for quantitative and qualitative determination of metals in solution. AAS utilizes the fact that each element absorbs and emits characteristic wavelengths of light (Lampman, 2010). The element is first atomized for instance in a flame and illuminated by light with a wavelength characteristic for the sample. For example, if silver measurements are to be performed on a solution a silver cathode lamp is used. A monochromator is used to reduce the effect of emission from the atomizer (Harris, 2007). A beam of light with a specific wavelength for the element to be detected is focused through an atomizing flame (flame AAS) or a quartz cuvette if the element to be analyzed is volatile (flameless AAS). The solution with the element to be detected enters the flame. The element to be de-

tected absorbs some of the light, thus reducing the intensity of the beam of light. A detector measures the change in intensity, converts this into absorbance and the concentration of the element is obtained by using Lambert-Beers Law. AAS measures total amount of silver atoms in solution. Hence, AAS does not differentiate between silver ions and silver atoms since an ionization buffer is usually added to keep the silver in atomic form. AAS is suitable for all solutions, provided that an appropriate sample preparation has been performed prior to analysis. A graphite furnace (GFAAS) can be used together with AAS to increase the sensitivity of the instrument (Harris, 2007).

ICP-OES

Inductively coupled plasma optical emission spectroscopy is an analytical technique used for detection of elements in liquids, particularly applicable to metals. ICP-OES uses argon to produce excited atoms and ions that emits electromagnetic radiation with wavelengths that are characteristic of a particular element. The concentration of the element can be determined from the intensity of the emission. The solution is pumped through a nebulizer which atomizes the solution into an aerosol in a spray chamber (Lampman, 2010). A part of the aerosol is introduced to a plasma flame created from argon gas. When the element of interest reaches the plasma it dissociates into atoms, ions and electrons due to the high temperature of approximately 8000°C in the plasma. Owing to the extremely high temperatures the atoms, ions and electrons collide with each other, resulting in excitation and de-excitation (Lampman, 2010). From the latter, light is emitted which is characteristic for each element. By knowing the intensity of these emissions and at what wavelength they occur the concentration of the element can be determined. ICP-OES is an advanced technique and measures total amount of silver in the solution. However, it does not give information on how much of the total amount of silver measured that was silver ions in the matrix. ICP is suitable for all solutions, provided that an appropriate sample preparation has been performed prior to analysis. Matrix effects on the yield of ions in the plasma are significant, therefore calibration standards should be in the same matrix as the unknown (Harris, 2007). It is also a very sensitive technique with a detection limit in the region of µg/L. In the experiments carried out for silver release from silver-containing dressings, the sensitivity obtained with ICP-OES is generally sufficient. Compared to AAS, ICP provides a higher sensitivity, a larger linear range and is a robust method (Lampman, 2010).

ICP-MS

This technique uses inductively coupled plasma as described in the previous section together with a mass spectrometry detector that separates the ions according to their mass-to-charge ratio. Compared to ICP-OES, ICP-MS provides higher sensitivity and is often used for measuring

trace elements in the region of ng/L. ICP-MS is also suitable when small amounts of samples are to be analyzed that has to be diluted (Lampman, 2010).

Due to the reactive nature of silver ions, different problems may arise when measuring silver. Silver may prove to be insoluble under certain conditions, it readily forms precipitates with chlorides and binds to thiol groups on proteins. In this project, silver release will be measured using a matrix that contains proteins and salts, thus simulating the wound environment to a large extent. This will however create problems when silver ions bind to chlorides, thus forming silver chloride precipitates, see reaction 1 in Figure 13. To avoid this problem, an excess of hydrochloric acid is used which will result in the formation of $AgCl_2^-$ (see reaction 2 in Figure 13). Also, since hydrochloric acid is in extreme excess the concentration ratio between H^+ and Ag^+ results in a negligible loss of Ag^+. Therefore, the probability that Ag^+ will bind to a negatively charged surface is insignificant. Nitric acid is used to oxidize and degrade the organic compounds in the matrix.

3. Development of release method

In this chapter the development of the release method is presented together with the selection of acceptor compartment and donator compartment.

3.1 Principle of the method

Plastic rings made of polyoxymethylene (POM) were manufactured at Mölnlycke, see Figure 4 and Figure 5. A semi-solid matrix was poured into the rings and was left to gel. A piece of dressing of a suitable size was pre-wetted with test fluid to mimic the conditions in the wound and was put on top of the semi-solid matrix. A weight was put on the dressing to ensure that the dressing was in contact with the acceptor compartment. The plastic ring with matrix, dressing and weight was put into a Petri dish to obtain a closed system and was incubated at 35°C for 24 hours, see Figure 6 for a schematic illustration of the set-up. The semi-solid matrix was put into a plastic container and digestion of the matrix was performed with hydrochloric acid and nitric acid. Silver determination was performed with ICP-OES.

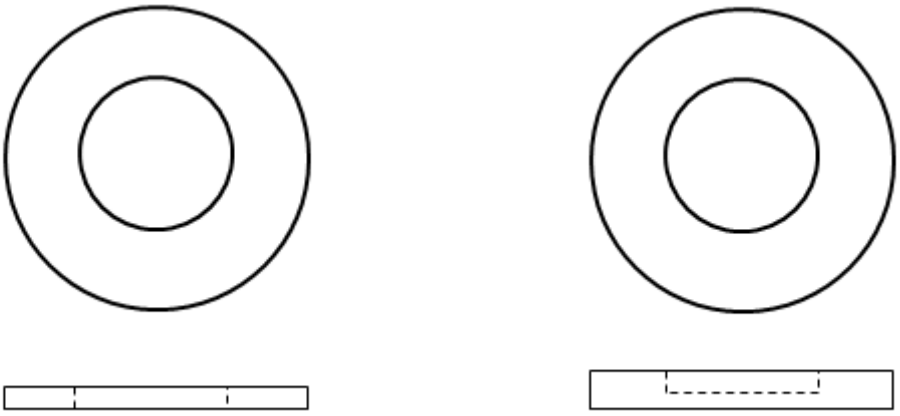


Figure 4 Schematic illustration of 1 mL POM ring without bottom (left illustration) and with bottom (right illustration).

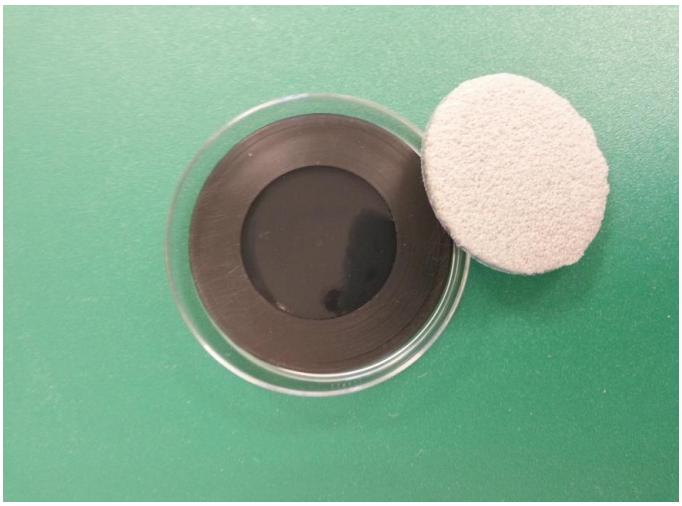


Figure 5 Acceptor compartment consisting of agar in POM ring equipped with bottom and piece of Mepilex® Ag dressing.

For all experiments in this work the incubation time and temperature was set to 24 hours and 35°C respectively. A time of 24 hours was chosen to allow silver release from the dressing and to have a reasonable working time. Incubation was performed at 35°C to simulate the temperature in the wound.

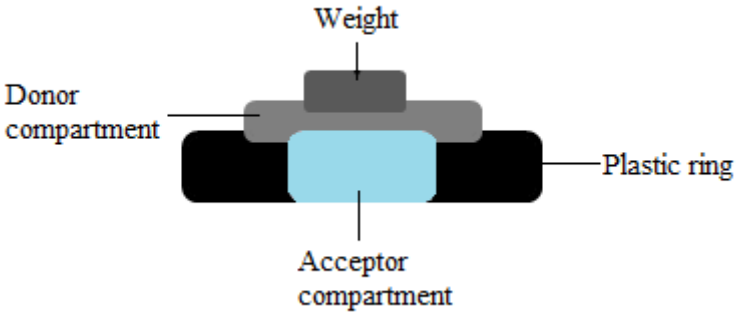


Figure 6 Schematic illustration of the set-up of the plastic ring (without bottom) with acceptor compartment, dressing and weight.

3.2 Acceptor compartment

In this section the choice of acceptor compartment including which type of gelling agent and concentrations are presented. A semi-solid matrix is necessary in order to determine silver release from dry or partly wetted products and material. The acceptor compartment was decided to contain approximately 50% fetal calf serum and approximately 50% saline solution to simulate the wound environment.

3.2.1 Choice of gelling agent

The method requires a semi-solid matrix in order to measure silver release. In this project three different types of gelling agents were examined; agar, collagen and gelatin. The gelling agents are to create a semi-solid matrix to be able to determine release from dry or partly wetted products

and material. Also human skin was tested as a matrix, to evaluate the relevance of the method being developed.

- *Agar* is a gelatinous substance derived from red algae. Agar contains agarose, a polysaccharide, and agaropectin. Agar is widely used in microbiology as a gelling substance and, together with nutrients, provides a solid media and a growth medium for bacteria and fungi. Concentrations tested were the following: 8 g/L, 10 g/L, 12 g/L.
- *Collagen* is a naturally occurring protein in mammals and is the main component of connecting tissue and the skin (Mathews, K. C., van Holde, K. E., Ahern, K. G., 1999). Concentrations tested were the following: 2 mg/mL, 2.39 mg/mL, 2.75 mg/mL and 4.6 mg/mL.
- *Gelatin* is a hydrolyzed form of collagen and consists of approximately 90% proteins, 10% water and only traces of salts. Concentrations tested were the following: 15 g/L, 20 g/L, 25 g/L, 30 g/L and 40 g/L.

3.2.2 Concentration of gelling agent

Preparation of agar as gelling agent

For an agar matrix solution with a concentration of 8 g/L, 10 g/L and 12 g/L, 0.8 g, 1.0 and 1.2 g respectively of agar-agar powder was weighed in a sterile flask. 50 mL saline solution (0.85% NaCl) was added and the solution was heated in microwave until the agar powder was dissolved and the solution became clear. The agar solution was put in a water bath (approximately 44°C) for one hour. 10 mL of fetal calf serum was heated in water bath (approximately 44°C) for 5 minutes and then mixed with 10 mL of agar solution in a centrifuge tube. For the small plastic rings 1 mL of the agar matrix solution was added and for the large plastic rings 2 mL of agar matrix solution was added to each ring.

Preparation of collagen gel as gelling agent

For 10 mL collagen matrix solution (2 mg/mL, 2.39 mg/mL, 2.75 mg/mL and 4.6 mg/mL collagen type 1), 2.2 mL, 2.6 mL, 3.0 mL and 5 mL respectively of collagen stock solution (9.18 mg/mL) was mixed with 1 mL acetic acid (0.1 vol.%), 6 mL SWF and 1 mL NaOH (0.1 M). The solution was kept on ice until use to prevent polymerization of the collagen. For the small plastic rings 1 mL of collagen matrix solution was added and for the large plastic rings 2 mL of collagen matrix solution was added to each ring. The rings with collagen matrix solution were incubated for 60 minutes at 35°C to allow for collagen polymerization.

Preparation of gelatin as gelling agent

For a gelatin matrix solution with a concentration of 15 g/L, 20 g/L, 25 g/L, 30 g/L and 40 g/L, 1.5 g, 2.0 g, 2.5 g, 3.0 g and 4.0 g respectively of gelatin powder was weighed in a sterile flask. 50 mL saline solution (8.5 g/L NaCl) was added and the gelatin solution was heated in microwave until the gelatin powder was dissolved and the solution became clear. The gelatin solution was allowed to cool down in a water bath (approximately 44°C) for one hour. 10 mL of fetal calf serum was heated in water bath (approximately 44°C) for 5 minutes and then mixed with 10 mL of gelatin solution in a 50 mL centrifuge tube. For the small plastic rings 1 mL of the gelatin matrix solution was added and for the large plastic rings 2 mL of gelatin matrix solution was added to each ring.

It was important to determine an appropriate concentration of acceptor compartment. To prevent the dressing absorbing moisture from the acceptor compartment thereby changing the degree of moisture saturation in the dressing it was important to have a sufficiently high concentration of the gelling agent in the acceptor compartment. However, since the developed method may be used in future microbiological studies a too high concentration of the acceptor compartment results in difficulties in decomposing the acceptor compartment mechanically (lumps of the acceptor compartment is not desirable for this purpose). In order to determine the optimal concentration of the gelling agent different concentrations were tested, see section 3.4. Different concentrations of gelling agents were prepared and poured into the plastic rings with a volume of 1 mL. Dry pieces of Mepilex® Ag (Ø31 mm) and Mepilex® Ag pieces that were pre-wetted with SWF to obtain a moisture saturation of 50% were placed on separate acceptor compartments. Four replicates were made for each concentration of agar. The pieces of dressing were weighed before placing them on the acceptor compartment. The plastic ring with acceptor compartment and the piece of dressing were put in a Petri dish and incubated for 24 hours at 35°C. After 24 hours the piece of dressing was weighed and the amount moisture absorbed by Mepilex® Ag was calculated.

3.2.3 Size and volume of acceptor compartment

Different parameters had to be considered when setting the dimensions of the acceptor compartment. The acceptor compartment should not be too big since the whole compartment was to be digested prior to analysis with ICP-OES. In addition, the acceptor compartment should be of manageable size and since the existing digestion method at Mölnlycke is suitable for a sample weight of 0.2–1 g, a smaller ring was manufactured which resulted in an acceptor compartment with a volume of 1 mL. However, the acceptor compartment should not be too small since the silver content had to be above the detection limit of ICP-OES. Therefore, to increase the detec-

tion limit a larger ring was also manufactured which resulted in an acceptor compartment with a volume of 2 mL. Digestion of this volume was proven to be sufficient. The dimensions of the acceptor compartments used were as following. For the acceptor compartment with a volume of 1 mL the inner diameter of the ring was 20 mm with an outer diameter of 40 mm. The thickness of the acceptor compartment was 3 mm. For the acceptor compartment with a volume of 2 mL the inner diameter of the ring was 30 mm with an outer diameter of 50 mm. The thickness of the acceptor compartment was 2.8 mm. Also, some rings were equipped with a bottom of a few millimeters and some did not have a bottom, see Figure 4.

3.2.4 Human skin as acceptor compartment

Assessment of silver release on a piece of human skin was performed to evaluate the relevance of the method being developed. Skin explants were obtained from a patient undergoing breast reduction surgery. Skin explants were kept in saline at maximum 8°C until use. The piece of skin was punched and cut into Ø12 mm large circles. Pieces of Mepilex® Ag was punched into Ø14 mm large circles to ensure that the entire skin piece was in contact with the dressing. Dry pieces of dressing and pieces pre-wetted with SWF to obtain a moisture saturation of 50% and 100% were placed on the piece of skin with a weight on top to ensure contact between skin and dressing. Each level of moisture saturation was performed in triplicate. The skin with dressing and weight was put in a Petri dish and incubated for 24 hours at 35°C. When human skin was used as acceptor compartment the rings were not needed.

3.3 Donator compartment (dressing/material)

In this section the dressing used is presented as well as the size of the dressing and the pre-wetting of dressing.

3.3.1 Dressing used

Mepilex® Ag was chosen as model dressing to be used during the development of the release method. The dressing consists of a silicone layer on polyurethane foam containing silver sulfate, see Figure 7. Mepilex® Ag is an absorbent dressing and has a backing consisting of waterproof and vapor permeable film. The dressing has a silver content of 1.2 ± 0.2 mg/cm^2. Batch 11438332 with expiry date 2013-10 was used for silver release measurements.

Figure 7 Mepilex® Ag.

3.3.2 Size of donator compartment

The size of dressing was chosen to cover the entire acceptor compartment and to create an overlap, see Figure 6. This overlap was to ensure that the total amount silver measured from the acceptor compartment was released from the surface of dressing intended for the wound. Therefore, test pieces of dressing were punched into Ø31 mm large circles for the acceptor compartment with a volume of 1 mL and Ø38 mm large circles for the acceptor compartment with a volume of 2 mL giving approximately 5 mm overlap on each side, see Figure 6.

3.3.3 Pre-wetting of dressing and used fluids

Pre-wetting of dressing was performed to simulate the usage of the dressing and to measure silver release at different degree of moisture saturation. There are a large number of silver-containing dressings on the market which vary significantly in terms of texture and their ability to absorb wound exudates. The moisture levels are therefore highly dependent on the materials or dressings being tested.

The dressings were pre-wetted before placing the dressing on the acceptor compartment. Three different media have been used in this project for pre-wetting the test pieces.

- Simulated Wound Fluid (SWF) which consists of fetal calf serum mixed with equal amount of peptone water. This media is used for simulating the environment in wounds. SWF contains salt, proteins, carbohydrates, amino acids, vitamins and other trace elements. Reports indicate that the total protein concentration of wound fluids collected from chronic wounds and acute wounds is dependent on many individual factors and can vary significantly; however, generally accepted protein values for both chronic and acute wound exudates are up to 50% of the total protein level of serum (Trengove et al. 1996, Aiba-Kojima et al. 2007)
- Solution A which consists of distilled water with NaCl (8.298 g/L) and $CaCl_2 \cdot 2H_2O$ (0.368 g/L). Solution A has the same ionic strength as body fluids and SWF.

- Deionized water. Used for Ag release determination from wound care products (Cavanagh et al. 2010, Parson et al. 2005, Wright et al. 1998)

To be able to measure silver release at different degree of moisture saturation it was important to know what volume to add to each dressing piece. The maximum amount of test fluid was measured and thereafter the amount of fluid to be added to obtain a certain degree of moisture was calculated. To obtain different moisture levels with the test fluids used, see Table 1. For a 0% moisture a dry piece of dressing was used. Test fluid was added by carefully pressing down the tip of the pipette on the wound contact surface of the dressing. To ensure that the test fluid was distributed evenly in the dressing the tip of the pipette was taken off and carefully rolled over the dressing. As can be seen in Table 1, the maximal absorption capacity of the product depends on the type of test fluid used and size of dressing.

Table 1 Weight of maximal absorption (given in grams) of two sizes of Mepilex® Ag with three different test fluids.

SWF		Solution A		H_2O	
Ø31 mm	Ø38 mm	Ø31 mm	Ø38 mm	Ø31 mm	Ø38 mm
4.2	7.4	4.0	6.7	4.2	6.7

To obtain a moisture saturation of 75%, 50% and 25%, the maximal absorption was multiplied with 0.75, 0.5 and 0.25 respectively. The dressing was then pre-wetted to the calculated weight.

3.4 Results and discussion of release method

3.4.1 Acceptor compartment

When choosing a suitable gelling agent for the acceptor compartment collagen, gelatin and agar were evaluated. It was of great importance to obtain a suitable concentration of the acceptor compartment. A sufficiently solid matrix was not obtained with collagen, although the highest concentration of collagen available on the market was used. When placing a piece of dressing on the acceptor compartment with collagen, the dressing absorbed most of the moisture resulting in change of moisture saturation inside the dressing. In addition, since the acceptor compartment was absorbed by the dressing a very small amount, only approximately 6% of the original weight of the acceptor compartment for a concentration of 2.39 mg/mL collagen remained for silver release measurements. Consequently, collagen was not used as acceptor compartment in this work. However, since collagen resembles the skin to a great extent it would be interesting to use collagen as acceptor compartment if a higher concentration of collagen was available.

Due to the low solidifying temperatures of gelatin (below 35°C), the acceptor compartment melted after incubation after merely some minutes. In this work a temperature of 35°C was set

to simulate wound environment and usage of dressing and for that reason the temperature was not tested in this thesis. Since gelatin melted at the chosen temperature, gelatin was not used as acceptor compartment in this work.

Compared to gelatin, agar was proven to be a more useful solidifying agent due to the better solidifying temperatures and it is available in concentrations that make a hard gel (comparison to collagen). Agar-agar powder mixed with FCS provided proteins and salts to the acceptor compartment, thus simulating the wound environment.

As described in section 3.2.2 an appropriate agar proportion in the AC was needed in order to measure silver release at different degree of moisture saturation. A low agar proportion in the AC results in larger absorption of moist to the dressing, thereby changing the degree of moisture saturation in the dressing. A too high agar proportion in the AC may result in transfer of test fluid in the dressing to the AC. Three different concentrations of agar as gelling agent were evaluated, 8 g/L, 10 g/L and 12 g/L. For the result, see Figure 8. An equal amount of absorption and desorption was preferred, therefore a concentration of 8 g/L was chosen.

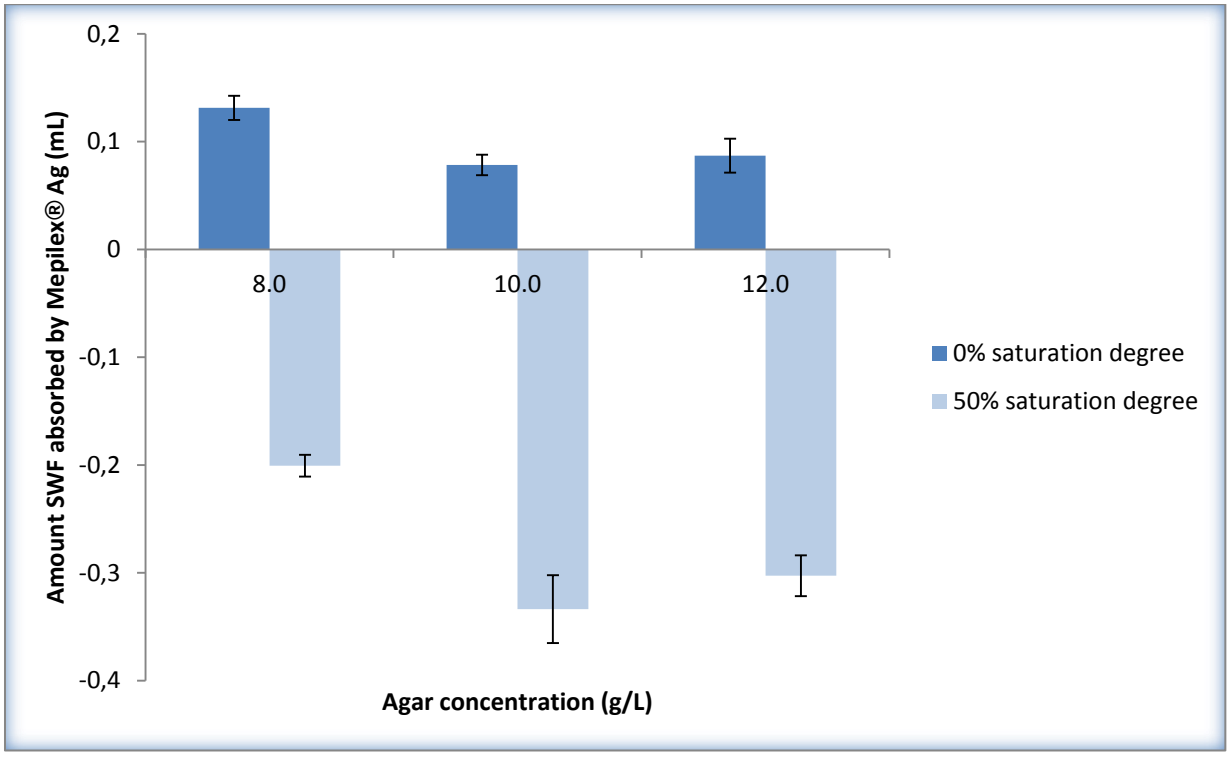


Figure 8 Amount SWF absorbed by Mepilex® Ag at different agar compositions. Error bars represent standard deviation. n=3

From the beginning, plastic rings were made without bottoms for easier removal of AC after incubation. Later on, experiments were also performed on rings equipped with bottoms. This resulted in a closed system and since the developed method may be used in the future for culturing

bacteria it was preferred to have a closed system for this purpose. Furthermore, there were no difficulties upon removal of the acceptor compartment in neither of the two types of plastic rings. The rings with a volume of 2 mL were produced to increase the detection limit of ICP-OES. Results showed that silver release could be detected from both rings. However, a precipitation after digestion could be seen for the larger AC (see chapter 4), and therefore the smaller ring was chosen. In Figure 9 silver release measurements from 1 mL and 2 mL rings with and without bottom are presented. The higher silver release from rings without bottom might be explained by a capillary attraction between the AC and the Petri dish.

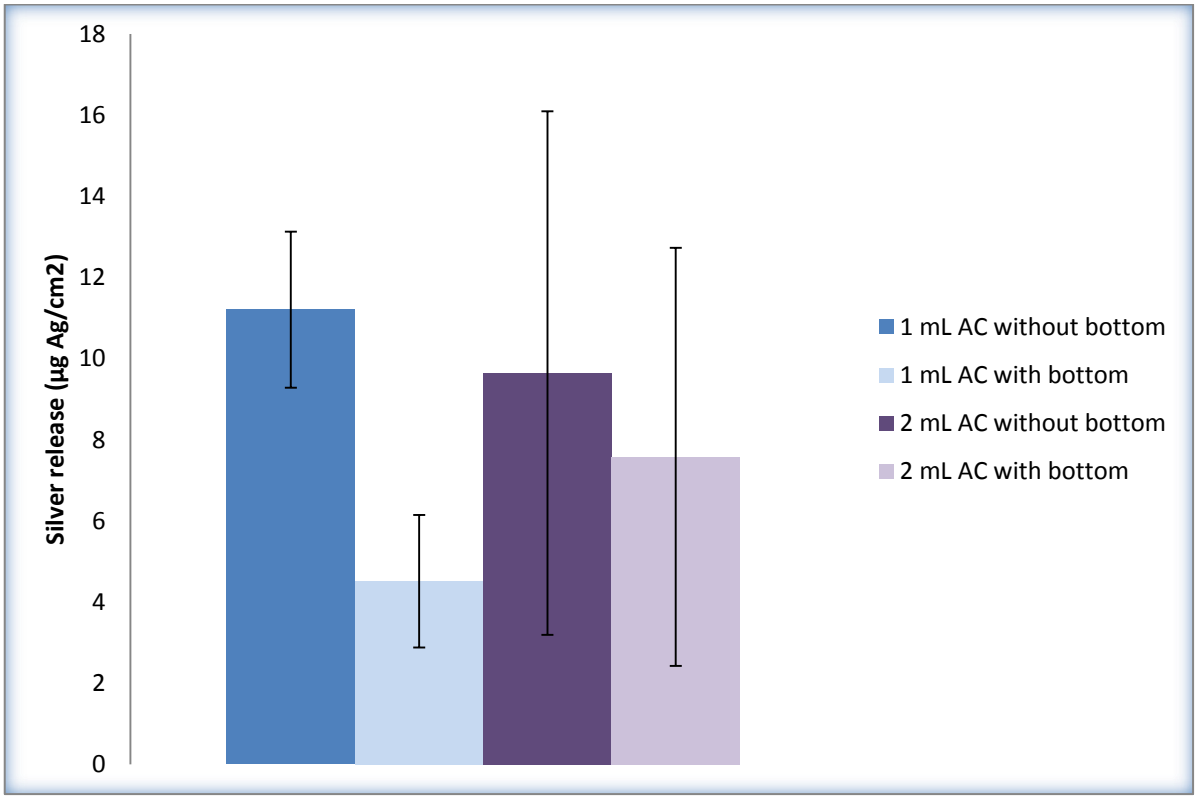


Figure 9 Silver release in µg Ag/cm2 measured from Mepilex® Ag on agar pre-wetted with SWF to 100% saturation. Error bars represent standard deviation. n = 4.

When comparing agar with skin as acceptor compartment, see Figure 10, good agreement regarding the silver release was obtained. This implies that the method developed is relevant for the analysis of silver release in a wound like environment. It also implies that agar as acceptor compartment can work well as a matrix that simulates wound like conditions. Although the experiment with skin was performed only one time, it gives an indication that agar can be used instead of skin. In addition, the use of skin raises questions about ethical issues as well as the risk of infection. Also, skin from different people can differ. The use of agar as acceptor compartment means more control and reproducibility of the acceptor compartment.

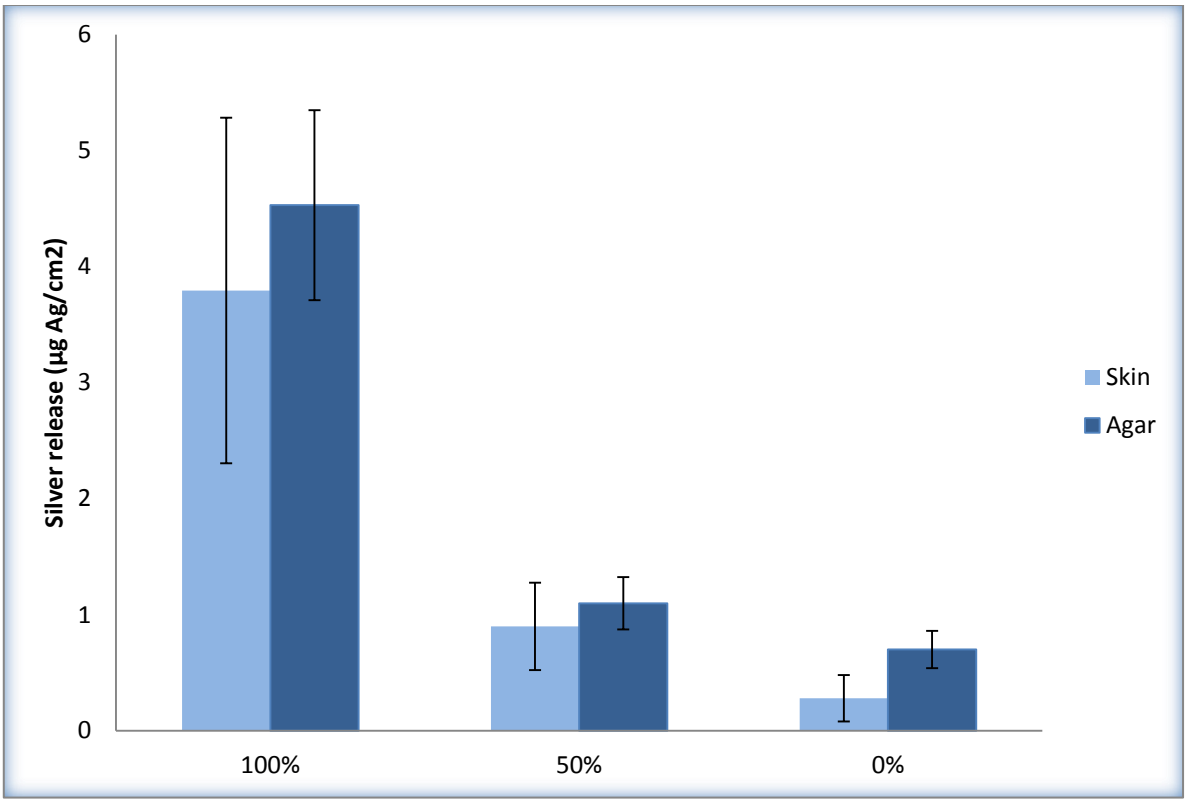


Figure 10 Silver release from AC consisting of skin and agar at different degree of moisture saturation with SWF. Error bars represent standard deviation. n = 4 for agar and 3 for skin.

3.4.2 Pre-wetting of dressing

It was difficult to get the test fluid evenly distributed on the wound contact surface on Mepilex® Ag. This was solved by carefully rolling the tip of the pipette over the piece of dressing.

Three different test fluids were used for pre-wetting of Mepilex® Ag, see Figure 11, namely SWF, solution A and deionized water. As can be seen the silver release is highly dependent on the type of test fluid used which is in agreement with Walker et al. 2006, Rigo et al. 2012 and Lindsay et al. 2010. The highest silver release is obtained with deionized water with a release of approximately 19 µg Ag/cm^2 AC. Dressing pre-wetted with solution A results in the lowest silver release, only about 1 µg Ag/cm^2 AC. SWF gives a silver release of approximately 4 µg Ag/cm^2 AC.

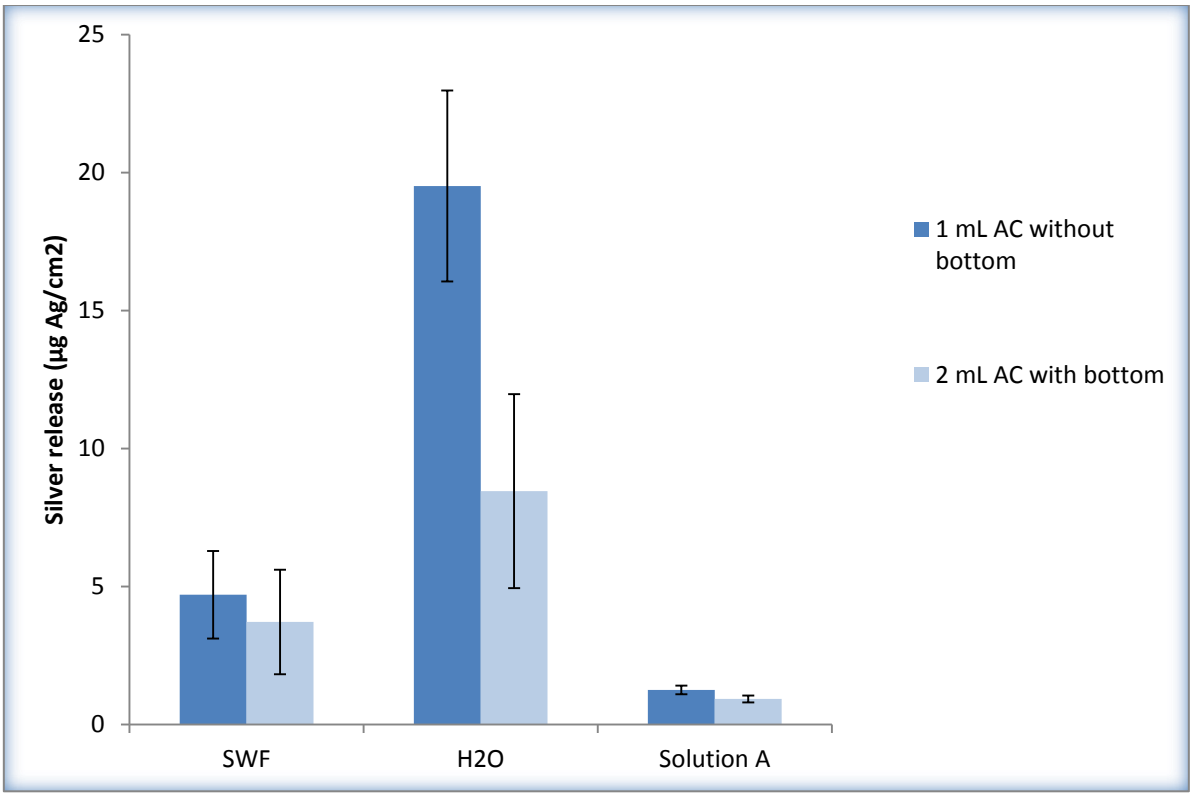


Figure 11 Silver release dependent on type of fluid used for pre-wetting. Experiment performed on 1 mL AC without bottom and 2 mL AC with bottom with agar as gelling agent. Error bars represent standard deviation. n= 4.

According to Walker et al. (2006) and Rigo et al. (2012) silver release is strongly dependent on the type of test fluid used for pre-wetting of dressing. The lowest silver release is obtained when pre-wetting the dressing with solution A which is a saline. Since solution A contains salts and no proteins the silver ions will presumably bind to the salts forming precipitates inside the product and will therefore not be released from the dressing, resulting in a low silver release. Rigo et al. (2012) showed in their study that high Cl^- concentrations tend to inactivate the dressings, thus reducing silver release from the product.

Pre-wetting with SWF, which is a fluid that contains both salts and proteins, leads to silver ions binding to both salts and proteins. A possible explanation for the higher silver release in SWF compared to solution A can be that the silver ions being bound to proteins is still in solution and is therefore able to be released (Rigo et al. 2012). However, silver bound to salts forms precipitates in the same way as with solution A and are therefore not released from the product. Therefore, the equilibrium is pushed so that silver will bind to proteins and therefore making the silver available for detection.

Water represents a simple dissolution medium and is not physiological appropriate as a model for wound exudates due to the lack of counter-ions (anions, such as chloride ions) that is normally present in exudates and which helps precipitate silver ions to form silver salts (Walker et al. 2006). Water was used as test fluid since studies have shown that Mepilex® Ag has the highest release of silver in water (Rigo et al. 2012). In their study it was shown that Mepilex® Ag released more than 50% of the total amount of silver within 5 minutes in ultra pure water. After 90 minutes, 96% of the total amount silver had been released. However, they used the beaker method as silver release method, see chapter 2.2, and the product was in excess of test fluid. The test fluid used was deionized water and silver could be released from the edges of the product. These parameters made it possible for such a high silver release. In the proceeding of this project SWF was chosen as test fluid since this simulates the wound environment to a better extent than deionized water or solution A.

As can be seen in Figure 11 there is a large difference in silver release between the two sizes of acceptor compartments pre-wetted with deionized water (19 µg Ag/cm^2 for 1 mL AC without bottom and 8 µg Ag/cm^2 for 2 mL AC with bottom). A possible explanation for the higher silver release in the smaller acceptor compartment may be the lack of bottom as discussed in section 3.4.1. There is a possible move of fluid under the plastic ring and the Petri dish, perhaps due to capillary forces, leading to an increased flow of silver in the AC resulting in a high silver release.

3.4.3 Silver release at different moisture levels

Silver release from Mepilex Ag was analyzed and the following results were obtained, see Figure 12.

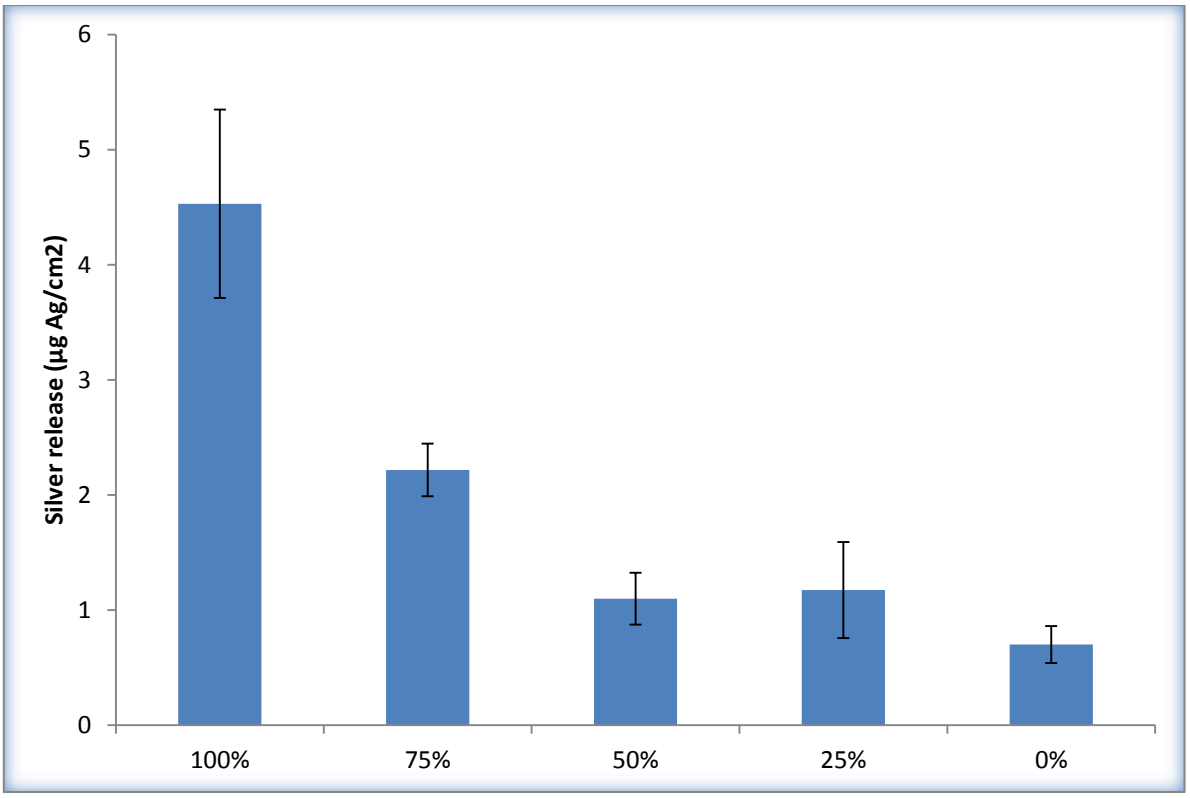


Figure 12 Silver release measurements at different degree of moisture saturation with SWF. Experiment performed on 1 mL AC with ring equipped with bottom. Error bars represent standard deviation. n= 4.

Experiments were performed in triplicates. A higher degree of moisture saturation accounted for a higher silver release. The developed method gives varying silver release at different degree of moisture saturation. Moreover, silver release could be detected and measured for dry pieces of dressings. However, there is no information on the amount silver that has to be present in the wound for antimicrobial effect. Therefore, most probably a sufficiently high liquid transport from the AC to the dressing may be necessary to obtain antimicrobial effect.

3.5 Conclusion of release method

To simulate the wound environment a suitable acceptor compartment needed to be evaluated with regard to gelling agent and concentration. Different gelling agents were analyzed, however agar with a concentration of 8 g/L was found to be most suited for the method being developed. Experiments performed on the different sizes of acceptor compartment and with/without bottom showed differences in regard to silver content. The larger ring with a volume of 2 mL was produced to increase the detection limit of ICP-OES. However, since silver release could be measured with the smaller ring, the ring with a volume of 1 mL equipped with bottom was chosen. To assess the relevance of the method being developed silver release on skin was analyzed and compared to the chosen acceptor compartment. The experiment showed good agreement with respect to silver release when comparing skin and agar with FCS as acceptor compartment.

Finally, measurements showed that silver release is highly dependent on the test fluid used for pre-wetting the dressing. The highest silver release was obtained with deionized water while the lowest amount of silver release was obtained with solution A. This behavior is probably caused by the presence/absence of proteins and salts. While the latter can bind with silver and form precipitates trapped inside the product, proteins that bind to silver may result in a higher silver release since these more likely will be in solution. Absence of proteins and salts means that nothing binds to silver, thus giving a higher silver release.

Measurements conclude that silver release depends on the moisture saturation of the dressing. The highest silver release was obtained for fully saturated pieces of dressings. The conclusion is that the developed method works well for analyzing silver release at different degree of moisture saturation.

4. Development of silver detection

In this section the digestion of acceptor compartment and human skin and the determination of silver release with ICP-OES are presented.

4.1 Silver detection

4.1.1 Digestion of acceptor compartment

After incubation, the acceptor compartments were transferred from the plastic rings into 50 ml plastic centrifuge tubes with screw cap and weighed. Pieces of human skin were treated in the same way. Thereafter 15 mL of hydrochloric acid (32% w/w) and 3 mL of nitric acid (65% w/w) were added and the centrifuge tubes were allowed to stand in room temperature for at least 48 hours. The screw caps were a bit loose during this period due to production of NO_2 gas, and then the caps were tightened. The containers with the acidic centrifuge tubes were transferred to a 65°C water bath for at least 4 hours and thereafter allowed to cool down. The content was later transferred to 50 mL volumetric flasks, the centrifuge tube was rinsed with deionized water and this was also transferred to the flask. The flask was filled with distilled water to the 50 mL mark. The content in the flask was analyzed within 2 days. In order to get a good calibration, matrix-matched standards were prepared by adding 0, 0.025, 0.1, 0.5 and 1 mL of Silver ICP Standard 1000 µg/mL to plastic centrifuge tubes containing 1 mL or 2 mL AC. This results in final calibration standard concentrations of 0, 0.5, 2, 10 and 20 mg/L respectively.

Digestion was performed with excess of hydrochloric acid to ensure that silver ions would form $AgCl_2^-$ (Reaction 2 in Figure 13). Nitric acid was also added and works as an oxidizer of organic material. Hydrochloric acid binds to the silver ions in the following way:

$$Ag^+(aq) + Cl^-(aq) \leftrightarrow AgCl\ (s)\ K_{sp} \text{ at } 25°C\text{: } 1.77 \times 10^{-10}\ M^2$$
$$AgCl\ (s) + Cl^-\ (aq) \leftrightarrow AgCl_2^-\ (aq)$$

Figure 13. Main silver reactions during digestion of matrix.

4.1.2 FTIR analysis

A precipitation could be seen for both 1 mL and 2 mL acceptor compartment after digestion. However, the precipitation was more evident for the acceptor compartment with a volume of 2 mL. There was a concern that this precipitate could contain silver that was not fully dissolved in the acid and FTIR analysis was performed to determine the content of the precipitate. Before analysis with FTIR the content in the flask was filtered using a glass fiber filter paper that could withstand the acidic solution. The precipitate appeared only as a small yellow discoloration on

the glass fiber filter paper. A small piece of the filter paper with precipitate was put on a diamond crystal, attenuated total reflectance (ATR), and analyzed. FTIR analysis was performed at Chalmers University of Technology with a Nicolet 6700 from Thermo Scientific with a DuraSamplIR II diamond ATR from SensIR Technologies. However, the database at Chalmers was inadequate for identification of the spectrum so a database search was performed at Mölnlycke using a Scientific Nicolet iS5 FTIR spectrometer equipped with ATR.

Infrared spectroscopy is a useful technique used to identify and study chemicals. Different molecules will absorb specific frequencies depending on the structure of the molecule when passing a beam of infrared light on it. FTIR imaging is useful tool when studying the distribution of chemical species with different molecular weights. With FTIR, vibrational transitions of a molecule can be detected and the benefit compared to conventional infrared spectroscopy is that all wavelengths can be measured immediately and greater sensitivity can be achieved (Lampman, 2010). An absorbance spectrum is obtained where the molecular structure of the compound can be determined.

A beam splitter divides the incoming beam into two optical beams. One beam goes to a fixed mirror plane and returns to the beam splitter while the other beam goes to a moving mirror plane (Harris, 2007). The motion in the mirror plane results in different pathlenghts. The two recombined beams are transmitted through the sample and the energy is absorbed due to the excited vibration in functional groups through a change in the dipole moment. The infrared signal that reaches the detector is measured. To analyze the signals the mathematic equation of Fourier transform is needed to calculate the spectrum. The computer plots the spectrum with absorbance versus wavenumber (Lampman, 2010).

4.1.3 SEM analysis

Scanning Electron Microscopy (SEM) analysis was together with FTIR used to determine the content of the precipitate. The same filter paper used for FTIR analysis was also analyzed with SEM with FEI Quanta 200 FEG-ESEM instrument. A small piece of the filter paper was put inside the SEM together with a piece of clean glass fiber filter paper as a reference. The analysis was performed with low vacuum mode.

SEM is a technique that uses electron beams to form an image. By utilizing the reversed photoelectric effect electromagnetic radiation is emitted from the surface when hit by electrons. The technique gives information such as chemical composition, morphology and crystalline structure of the sample (Swapp, 2012). Electrons are formed by heating up a tungsten filament and the electrons are focused into a small beam through one or two lenses. The beam with electrons

later hits the sample. The accelerated primary electrons interact with the atoms of the sample and lose energy by absorption. These incidents cause the electrons of the sample to be emitted by elastic and inelastic scattering events. The elastic scattering events results in backscattered electrons, which give information about the crystal structure and the inelastic scattering events results in secondary electrons. Detectors measure the backscattered electrons and secondary electrons, thus forming the image (Swapp, 2012).

4.1.4 Determination of silver content with ICP-OES

Amount silver in the 50 mL volumetric flasks were determined by ICP-OES at two wavelengths using axial mode in the range 0.02-20 mg/L, according to Table 2. The ICP-OES was calibrated with the five calibration standards 0, 0.5, 2, 10 and 20 mg/L described in section 4.1.1.

Table 2 Determination of silver release in solutions with ICP-OES.

Element	Wavelength	View type	Measuring range	Comment
Ag	328.068 nm	Axial	0.02-20 mg/L	Used to quantify Ag
Ag	338.289 nm	Axial	0.02-20 mg/L	Used to verify absence of interferences

4.1.5 Calculations

The silver concentration obtained from ICP is expressed in mg/L. This value is converted to mg silver/flask by multiplying with 0.05 because of the dilution step in the preparation for ICP analysis. To express the concentration in µg silver/cm^2, the area of the two different sizes of dressings needs to be calculated. For the acceptor compartment with Ø20 mm the area is calculated to 3.14 cm^2 and for the acceptor compartment with Ø30 mm the area is calculated to 7.07 cm^2. The piece of skin that was tested was punched into Ø12 mm so that the area became 1.13 cm^2.

4.2 Results and discussion of silver detection

It was found that the current method for digestion at Mölnlycke was suitable for digestion of the acceptor compartment. Still, a precipitation could be seen for both sizes of volume after digestion of the acceptor compartment. However, the precipitation was more evident for the AC with a volume of 2 mL. Since there was a concern that this precipitate could contain silver that was not fully dissolved in the acid FTIR analysis together with SEM analysis was performed to determine the content of the precipitate. Result from SEM analysis is presented in Figure 14. The red curve represents the precipitate while the yellow graph represents a reference filter paper that was analyzed since the precipitate was analyzed on a glass fiber filter paper. If silver would have been present a peak at 2.95 keV should have been obtained. Since no peak was obtained at that position it was concluded that no silver was detected in the precipitate with this method. Instead, SEM results conclude that the precipitate consists of carbon, chlorine and nitrogen. These elements likely originate from HCl salts of the proteins from the AC consisting of agar, saline and FCS. Due to the low pH in the solution, probably cations formed will attract negatively charged chloride ions which are in excess. The high concentration of these salts results in precipitation. Finally, all silver seem to be in solution. Therefore, it is not necessary to develop the digestion of AC further.

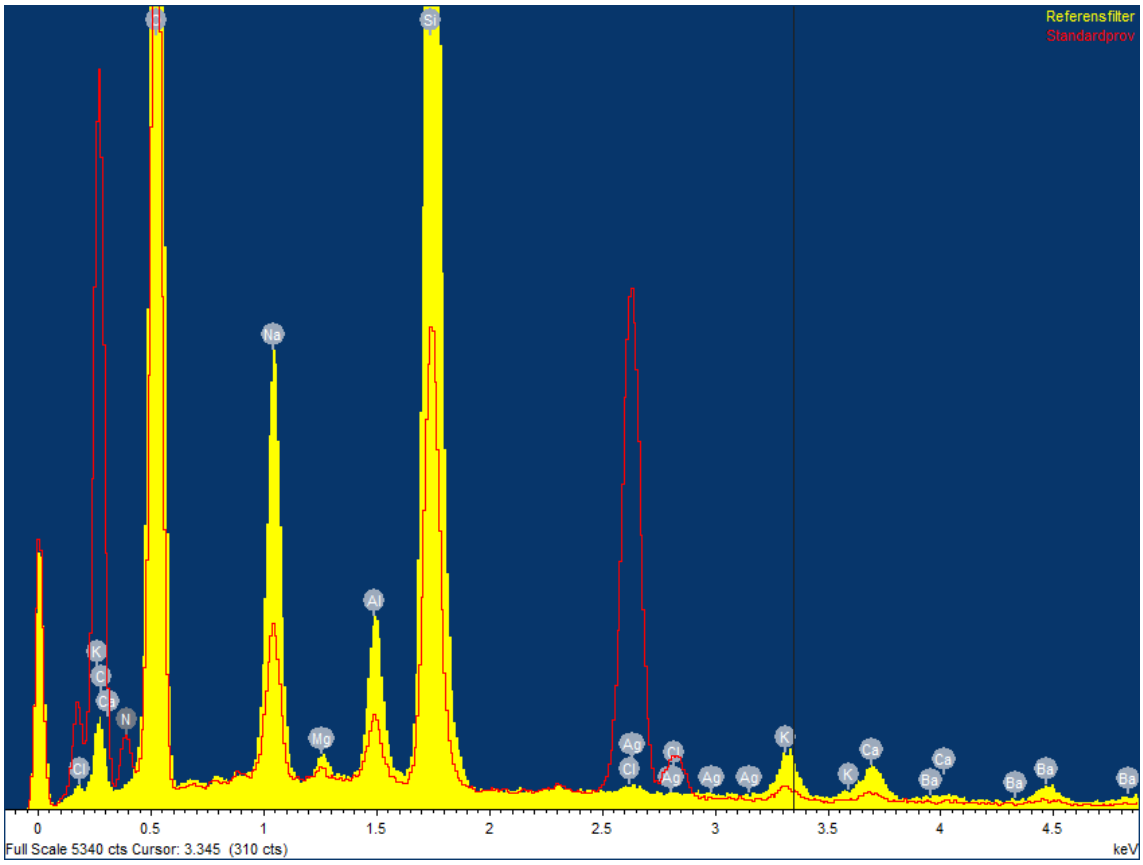


Figure 14 SEM analysis performed on the precipitation obtained from digestion of acceptor compartment. The red curve represents the precipitate while the yellow graph represents a reference filter paper.

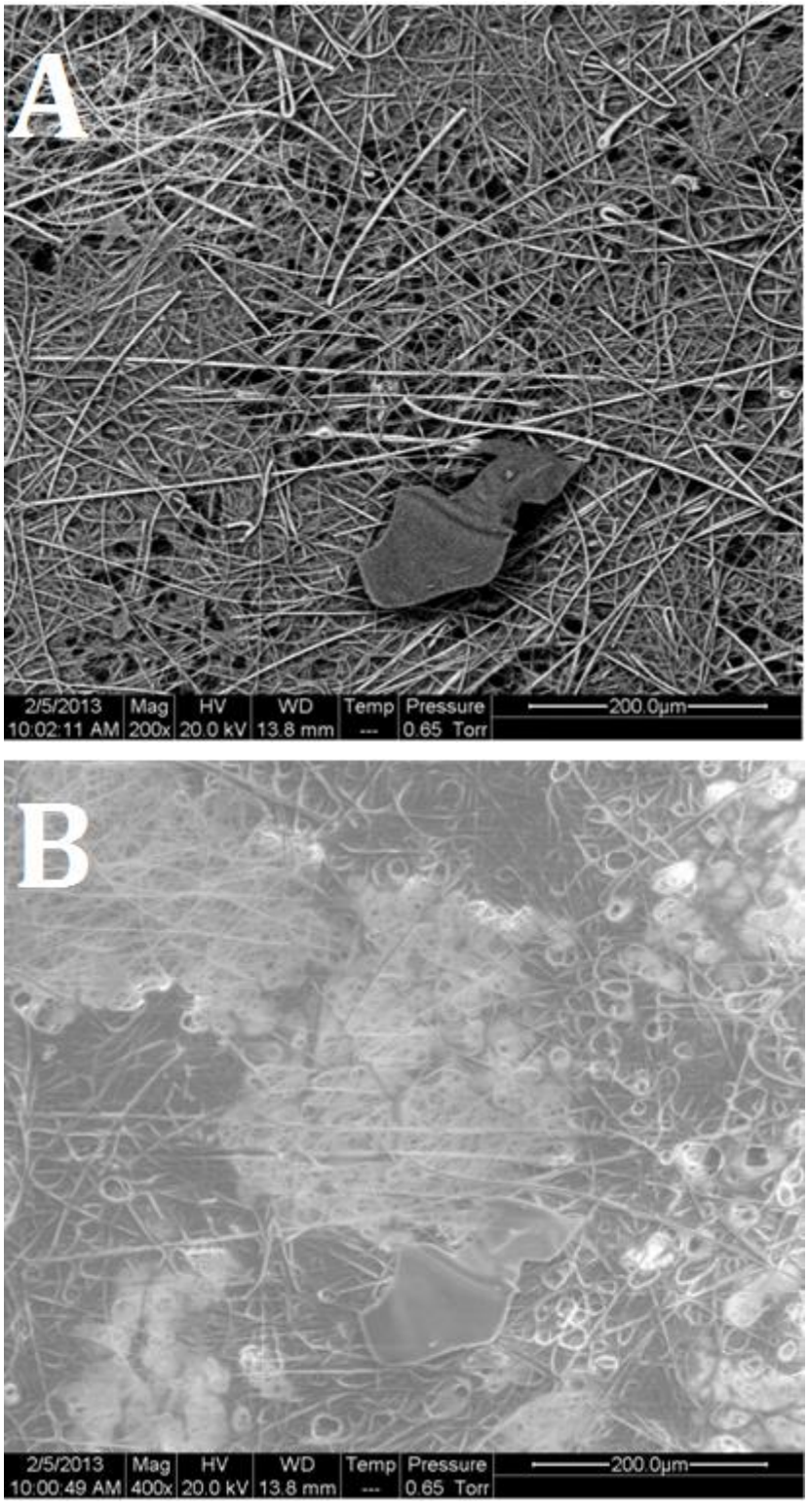


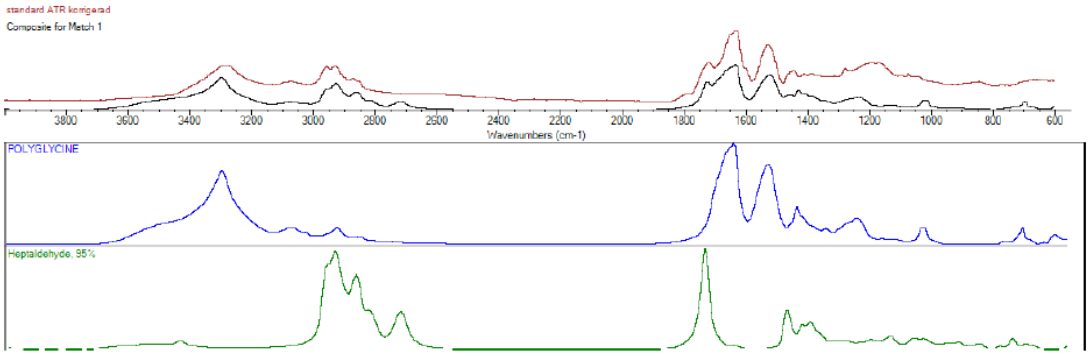
Figure 15 SEM picture from analysis of precipitation on glass fiber filter. Picture A obtained from backscattered electrons, picture B obtained from secondary electrons.

Pictures of the precipitation on a glass fiber filter was taken with SEM, see Figure 15. Picture A is obtained from the backscattered electrons, and picture B is obtained from the secondary electrons. The precipitation can be seen in picture B as a hazy deposit. In the pictures a large foreign

specimen can be seen in Picture A and SEM analysis was performed on the specimen. Large amounts of chlorine could be seen, and it was believed to be a contaminant, probably a small piece of polyvinyl chloride.

The result from FTIR analysis is presented in Figure 16. When the graph was compared with Omnic software database a multi-component search was performed. The result showed good agreement with the protein polyglycine. After a search in the protein database, see Figure 17, it was believed to be the peptide N-CBZ-glycyl-L-pro-L-leu-gly-L-proline which might originate from FCS and the possibility of this not being fully digested. The analysis of the precipitate with SEM and FTIR is in agreement with each other.

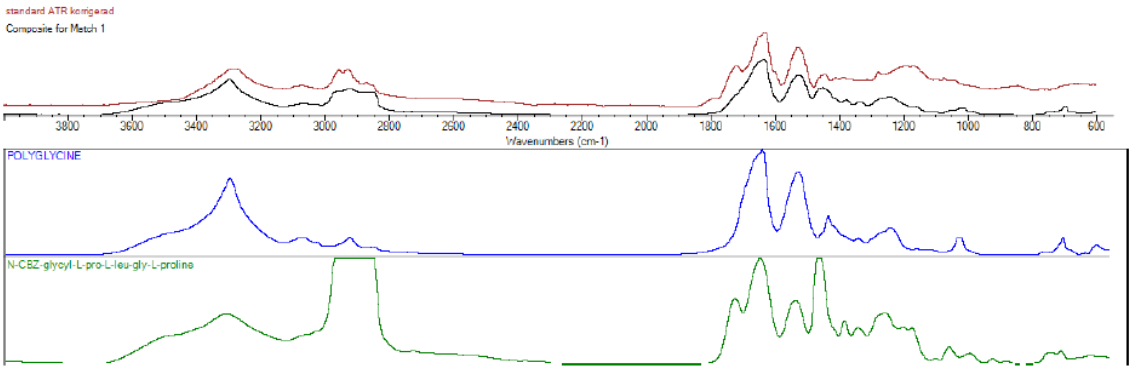
Multi-Component Search Results



	Match	Title	Cumulative	Composite%	Folder	Filename	Index
1	55.60	POLYGLYCINE	40.71	68.95	Aldrich FT-IR Collection Edition II	c:\my documents\omnic\libs\sea207.lbd	17925
		Heptaldehyde, 95%	55.60	31.05	Aldrich Aldehydes and Ketones	c:\my documents\omnic\libs\sea215.lbd	249

Figure 16 A multi-component search using FTIR for analyzing the precipitation.

Multi-Component Search Results



	Match	Title	Cumulative	Composite%	Folder	Filename	Index
1	59.21	POLYGLYCINE	40.71	63.67	Aldrich FT-IR Collection Edition II	c:\my documents\omnic\libs\sea207.lbd	17925
		N-CBZ-glycyl-L-pro-L-leu-gly-L-proline	59.21	36.33	Sigma Proteins and Peptides	c:\my documents\omnic\libs\sea240.lbd	186

Figure 17 A multi-component search using FTIR for analysis of the precipitation. This is compared to a protein database.

To verify the ICP measurement with regard to silver content a reference was prepared with an acceptor compartment with the gelling agent agar. A known amount of silver solution calculated to result in a silver content of 20 mg/L was added to the acceptor compartment and digestion was performed in the same way as the other release measurements. The measured silver content in the reference sample showed acceptable agreement with the silver added from the beginning (accuracy 112% with CV of 7.9%, n=6). The higher silver concentration obtained can be caused by something in the sample preparation.

4.3 Conclusion of silver detection

It was found that the current method for digestion at Mölnlycke was suitable for sample digestion of the acceptor compartment and determination of silver. The precipitation that could be seen was analyzed with SEM and FTIR and concluded that no silver was detected and that the precipitation consisted of an organic compound, possibly a protein originating from the AC.

5. Conclusion

Different types of gelling agents in the AC were investigated in this project and concluded that an AC produced from agar mixed with saline and 50% FCS was the most suitable. The relevance of the developed method was tested by using human skin as AC. Results showed good agreement with respect to silver release when skin and agar was compared as AC. Since a closed system was preferred due to possible future microbiological studies a ring equipped with bottom was chosen. The volume of the ring was selected to 1 mL. Measurements showed that silver release is highly dependent on the test fluid used for pre-wetting the dressing. The highest silver release was obtained with deionized water while the lowest amount of silver release was obtained with solution A. This behavior is likely caused by the presence/absence of proteins and salts. Salts can bind with silver and form precipitates which are trapped in the product and decrease silver release to the AC. Proteins that bind to silver are still in solution and can be released to AC which results in a higher silver release. Absence of proteins and salts means that nothing binds to silver, thus giving a higher silver release. Measurements performed at different degree of moisture saturations conclude that silver release depends on the moisture saturation of the dressing. The highest silver release was obtained for fully saturated pieces of dressings. The conclusion is that the developed method works well for analyzing silver release at different degrees of moisture saturation.

The acid digestion method currently used at Mölnlycke was suitable for digestion of the AC. Analysis performed by SEM and FTIR on the precipitation concluded that no silver was present and that all silver was in solution. Furthermore, the precipitation was considered consisting of an organic compound, possibly a protein originating from the HCl salts of the proteins from the AC.

Further work

As mentioned in section 3.1 some of the parameters were locked from the beginning and not evaluated in this project. Further studies could be done in order to investigate the effect of time on silver release. Since Mepilex® Ag can be used for up to 7 days it would be interesting to measure silver release for a longer time period than 24 hours.

Future studies may include investigation of the antibacterial effect of silver with the developed method, use of collagen together with agar and more studies performed on skin. It would be interesting to examine silver transfer in the different layers of the skin. Finally, more silver release measurements can be done with the developed method.

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References

Aiba-Kojima, Emiko, Tsuno, et al. (2007) Characterization of wound drainage fluids as a source of soluble factors associated with wound healing: comparison with platelet-rich plasma and potential use in cell culture. *Wound Repair and Regeneration*, vol. 15, no. 4, pp. 511-520.

Burrell, R. E. (2003). A Scientific Perspective on the Use of Topical Silver Preparations. *Ostomy Wound management,* vol 49, no. 5A, pp. 19-24.

Cavanaugh, M. H., Burell R. E., Nadworny P. L. (2010) Evaluating antimicrobial efficacy of new commercially available silver dressings. *International Wound Journal*, vol. 7, no. 5, pp. 394-405.

Dolmer, M. et al. (2004) In vitro silver release profiles for various antimicrobial dressings. Poster presented at the 2nd World Union of Wound Healing Societies' meeting in Paris, France, July 2004.

Hamberg, K., Jakobsen, C., Taherinejad, F., Kaszonyi, G. (2012) Correlation of silver release and antimicrobial effect of silver-containing wound dressing in vitro. Poster presented at 22nd ETRS-meeting, October 4-5 2012, Athens, Greece.

Hanson, W. A. (1991). *Handbook of dissolution testing*. USA: Aster Publishing Corporation.

Harris, D. C. (2007) *Quantitative Chemical Analysis*, 7th edition. New York: W. H. Freeman and Company.

Kebbekus, B. B. (2003) *Preparation of samples for metals analysis*. Mitra, S. (Eds) *Sample Preparation Techniques in Analytical Chemistry* pp. 227-264. *Department* of Chemistry and Environmental Science New Jersey Institute of Technology. A John Wiley & Sons, Inc., Publication.

Lampman, Pavia, Kriz, Vyvyan. (2010) *Spectroscopy.* Fourth edition. California. Brooks/Cole. pp. 24-25.

Lansdown A. B. G, Williams. A. (2004) How safe is silver in wound care? *Journal of wound care*, vol 13, no 4, pp. 131-136

Lansdown A. B. G. (2004) A review of the use of silver in wound care: facts and fallacies. *British Journal of Nursing*, vol. 13, no. 6. pp. 6-19.

Lavilla, I. (2007) Fast determination of arsenic, selenium, nickel and vanadium in fish and shellfish by electrothermal atomic absorption spectrometry following ultrasound-assisted extraction. *Food Chemistry*, vol 106 (2008), pp. 403-409

Lide, D. R. (1990) *Handbook of chemistry and physics.* 71st edition. USA: CRC Press, Inc.

Lindholm, C. (2003) Sår. Second edition. *Studentlitteratur,* Lund.

Lindsay, S., DelBono, M., Stevenson, R., Stephens, S., and Cullen, B. (2010) The silver release profile of antimicrobial wound dressings: standardizing *in vitro* evaluations. Poster presented at SAWC 2010.

Mathews, K. C., van Holde, K. E., Ahern, K. G. (1999) *Biochemistry*, 3rd edition. San Francisco: Addison-Wesley Publishing Company.

Parsons D., Bowler P. G.,Myles V., Jones S. (2005) Silver Antimicrobial Dressings in Wound Management: A Comparison of Antibacterial, Physical, and Chemical Characteristics, *Wounds*, vol. 17, no. 8, pp. 222-232.

Percival, S. L., Bowler, P. G., Russell, D. (2005) Bacterial resistance to silver in wound care. *Journal of Hospital Infection*, vol 60, pp. 1-7.

Priego-Capote, F., Luque de Castro, M.D. (2004) *Analytical* uses of ultrasound. Sample preparation. *Trends in Analytical Chemistry*, vol 23, no 9.

Rea, A. W., Keeler, G. J. (1998) Microwave digestion and analysis of foliage for total mercury by cold vapor atomic fluorescence spectroscopy. *Biogeochemistry,* vol 40, pp. 115-123.

Rigo, C. et al. (2012) Characterization and evaluation of silver release from four different dressings used in burns care. *Burns*, http://dx.doi.org/10.1016/j.burns.2012.06.013

Schwartzkopf, A., Brill, H., Buchholtz, C., Braunwart, H. (2010) Freisetzung von Silberionen aus Schaumverbänden. *Wund Management*, vol 4, no 1, pp. 10-13

Swapp, S. (2012) Scanning Electron Microscopy. *Geochemical Instrumentation and Analysis.* [Online] February 10, 2012.

The European Virtual Institute for Speciation Analysis. (2010) Uptake of arsenic through human skin depends strongly on its speciation. Retrieved 2013-02-13 from http://www.speciation.net/News/Uptake-of-arsenic-through-human-skin-depends-strongly-on-its-speciation-;~/2010/05/17/5019.html

Thomas, S. (2010) Silver Dressings. In: *Surgical Dressings and Wound Management*. Medetec Publications, Cardiff, South Wales.

Trengove, S. R. et al. (1996) Biochemical analysis of wound fluid from nonhealing and healing chronic leg ulvers. *Wound Repair and Regeneration*, vol. 4, no. 2, pp. 234-239.

US Pharmacopeia. (2006) Monograph 724 Drug Release, UPS29-NF24.

Walker, M., Cochrane C.A., Bowler, P. G., Parsons D., Bradshaw, P. (2006) Silver deposition and tissue staining associated with wound dressings containing silver. *Ostomy Wound Management*, vol. 52, no. 1, pp. 42-44, 46-50.

Wright, J.B., Hansen, D. L., Burrell, R. E. (1998) The comparative efficacy of two antimicrobial barrier dressings: in-vitro examination of two controlled release of silver dressings. *Wounds*, vol. 10, no. 6, pp. 179-188.

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