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APPLICATION OF LOW-COST FILTER PAPER COATED WITH SILVER NANOPARTICLE FOR Escherichia coli REMOVAL IN EMERGENCY SITUATION

MOHD IZDHAM BIN MOHD YUNI

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APPLICATION OF LOW-COST FILTER PAPER COATED WITH SILVER NANOPARTICLE FOR *Escherichia coli* REMOVAL IN EMERGENCY SITUATION

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BY

MOHD IZDHAM BIN MOHD YUNI

Thesis submitted in fulfilment of the requirement for the degree of Bachelor Science (Environmental and Occupational Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

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Thank you.

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ABSTRACT

APPLICATION OF LOW-COST FILTER PAPER COATED WITH SILVER NANOPARTICLE FOR *Escherichia coli* REMOVAL IN EMERGENCY SITUATION

MOHD IZDHAM BIN MOHD YUNI

Introduction: A number of water filter products have been invented to overcome unclean drinking water problems during emergency situation which involve contamination of water sources especially on biological contamination. However there are limitations in the readily made water filter product in the current market. Recent products of water filter invented by manufacturer tend to be high in cost. In emergency situation, the need for cheap and low-cost water filter product is vital. Other than that, some products come with complex instruction-to-use. In emergency situation, public need fast and easy access for clean drinking water. **Objectives**: The study aimed to test the performance of low-cost filter paper coated with silver nanoparticle based on two shapes (round and rectangular) with six ratios SDS/ $AgNO_3$ (0.5,2,5,10,15 and 20) to remove *Escherichia coli* from drinking water to be used during emergency situations. **Methodology:** The chemical reduction method has been used to coat cellulose filter paper with silver nanoparticles by using $NaBH_4$ and $AgNO_3$ with six difference ratios of SDS/$AgNO_3$ ranging from 0.5 to 20. Two difference shapes; round and rectangular shapes of filter paper have been tested. The performance of low-cost filter paper coated with silver nanoparticle for *Escherichia Coli* removal in emergency situation was tested using artificial water samples with 10^4 cfu/mL of *Escherichia coli.* To take into consideration of drinking water quality standards and non-carcinogenic health risks, the optimum SDS/$AgNO_3$ ratio for the cellulose filter paper coating was selected by comparing the silver in the effluent with *Escherichia coli* removal. **Results and Discussion:** For all six ratios from 0.5, 2, 5, 10, 15 and 20, 100% *Escherichia coli* removal was observed for both filter paper designs. This proved that SDS ratios used can protect the silver nanoparticle formed on cellulose filter paper during the filtration process. **Conclusion:** This study still need to consider other factors including field testing before final decision on the best ratio of SDS/$AgNO_3$ can be made. The best ratio of SDS/$AgNO_3$ cannot be determined in regard of no significant difference between ratio of SDS/$AgNO_3$ and silver concentration in effluent. The round shape of filter paper was determined as the best shape for filtration when it show the lowest mean of silver concentration compared to rectangular shape.

Keywords: Silver nanoparticles, *Escherichia coli (E. coli), low cost filter, emergency*

ABSTRAK

APLIKASI KERTAS PENAPIS KOS RENDAH DISALUTI NANOPARTIKEL PERAK UNTUK PENYINGKIRAN *Escherichia coli* DALAM SITUASI KECEMASAN

MOHD IZDHAM BIN MOHD YUNI

Pengenalan: Beberapa produk penapis air telah dicipta untuk mengatasi masalah air minuman yang tidak bersih dalam situasi kecemasan terutama yang melibatkan pencemaran biologi. Akan tetapi, terdapat beberapa kekurangan bagi produk penapis air yang berada di pasaran semasa. Produk penapis air yang dicipta oleh pengeluar adalah mahal. Dalam situasi kecemasan, penapis air kos rendah dan juga akses yang cepat dan mudah untuk air minuman yang bersih adalah penting untuk kegunaan mangsa. **Objektif**: Kajian ini bertujuan untuk menguji prestasi kertas penapis kos rendah disaluti dengan nanopartikel perak dengan dua bentuk (bulat dan segi empat tepat) dengan enam nisbah SDS/$AgNO_3$ (0.5,2,5,10,15 dan 20) untuk menyingkirkan *Escherichia coli* daripada air minuman yang akan digunakan semasa situasi kecemasan. **Kaedah kajian**: Kaedah pengurangan kimia telah digunakan untuk menyaluti kertas penapis selulosa dengan nanopartikel perak menggunakan $NaBH_4$dan $AgNO_3$ bersama enam nisbah SDS/$AgNO_3$ yang berbeza (0.5, 2,5,10,15 dan 20). Dua bentuk yang berbeza; bulat dan bentuk segi empat tepat kertas penapis telah diuji. Prestasi kertas penapis kos rendah disalutI dengan nanopartikel perak untuk penyingkiran *Escherichia coli* dalam situasi kecemasan menggunakan sampel air buatan dengan 10^4cfu / mL *Escherichia coli*. Standard kualiti air dijadikan perbandingan untuk memilih nisbah SDS/$AgNO_3$ yang terbaik bagi menyalut kertas penapis selulosa dengan nanopartikel perak dengan melakukan perbandingan kandungan perak dalam air tapisan dengan kadar penyingkiran *Escherichia Coli*. **Keputusan dan Perbincangan**: Untuk kesemua enam nisbah daripada 0.5, 2, 5, 10, 15 dan 20, 100% *Escherichia coli* disingkirkan untuk kedua-dua reka bentuk kertas penapis. Ini membuktikan bahawa nisbah SDS/$AgNO_3$ yang digunakan boleh melindungi nanopartikel perak yang terbentuk di atas kertas selulosa penapis semasa proses penapisan. **Kesimpulan**: Kajian ini masih perlu mengambil kira faktor-faktor lain termasuk ujian lapangan sebelum keputusan muktamad mengenai nisbah terbaik SDS/$AgNO_3$ boleh dibuat. Ratio terbaik tidak dapat ditentukan oleh kerana tiada signifikasi antara enam ratio SDS/$AgNO_3$ dengan jumlah perak dalam air. Bentuk bulat dipilih sebagai bentuk terbaik untuk penyingkiran *E.coli* kerana julat perak didalam air adalah paling sedikit berbanding bentuk petak.

Kata kunci: Nanopartikel perak, *Escherichia coli (E. coli)*, Kertas penapis kos rendah, kecemasan

TABLE OF CONTENTS

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CHAPTER 4: RESULT AND DISCUSSION

CHAPTER 5: CONCLUSION, STUDY LIMITATION AND RECOMMENDATION

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LIST OF TABLES

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LIST OF FIGURES

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LIST OF ABBREVATIONS

WHO	World Health Organization
SDS	Sodium Dodecyl Sulphate

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CHAPTER 1

INTRODUCTION

1.1 Background

Emergency situations can cause abundance of major problem. Among the problems arise from the emergency situations such as flood, tsunami, earthquake and etc., drinking water problems typically to be one of the most vital aspects to be considered. Sources such as rivers and wells across the areas being affected with the disaster will be physically, chemically and biologically contaminated. The contamination of water sources during the emergency situations causes limited access to the clean drinking water supplies. The World Health Organization (2016) mentions that floods contaminate freshwater supplies which will heighten the risk of water-borne diseases. Faecal contamination in clean water sources for instance, may be sporadic and the degree of faecal contamination may be fluctuate widely (World Health Organization, 1995). Water-borne pathogen such as *Escherichia coli* contamination in ambient water bodies and related diseases are the major water quality concern throughout the world (Pande et al., 2014). (Lechevallier et al., 1996) have highlighted that the public health poses a great danger when consuming unsafe water supply which can lead to outbreaks of diseases, such as cholera and gastroenteritis. In Malaysia perspective, Department of Health raised the alert for diseases such as cholera, typhoid and dysentery in the aftermath of the floods. Thus,

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it is crucial to provide a mean during emergency situations for clean potable drinking water.

Recently, nanotechnology has come out with a various types of nanomaterial which introduced to water treatment and water industries that can have promising outcomes. Xu et. al., (2012) and Ali, (2012) mentioned that the possibility of nanotechnology to incorporate in environmental applications has been widely examined during the last few years back as a way to improve efficiency of conventional treatment methods. Nanoparticles, as suggested by Praveena et al. (2016) have shown unique characteristic in term of physical, chemical and biological properties to be compared with macro-scaled particles. Simeonidis et al. (2015) stated that an obvious advantage of nanoparticles in water treatment was due to their characteristic which were significantly small in size and consequently the increased specific surface area compared to bulk materials which provides both high reactivity and uptake capacity. However, the use and application of nanotechnology in Malaysia is still low compared to other developed country. According to Hashim et al. (2009), nanotechnology in Malaysia is still at the beginning or early stage. Malaysia is still suffering from a few shortfalls regarding nanotechnology development such as no definitive plan to realize and develop nanotechnology industries, and lack of efforts to promote awareness in nanotechnology (Hashim & Nadia, n.d.).

1.2 Research problem

A number of water filter products have been invented to overcome the unclean drinking water problems during emergency situation which involved with the contamination of water sources especially on biological contamination such as faecal contamination. However there were some limitations need to be considered arising

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from readily made water filter product at current market. Recent products of water filter invented by manufacturer tend to be high in cost. Also, most of water filter product invented had to use power supply as for water filter to be function.

In emergency situation, the needs for cheap and low-cost water filter product are vital as people suffer from the disaster. Also, power supply during disaster will be limited and any equipment and product that have to use power supply will not be able to operate. In emergency situation, public needs for fast and easy access for clean drinking water.

1.3 Study Justification

Cellulose filter paper incorporation with silver nanoparticles is local made product which will benefit more for local use. The application of cellulose filter paper incorporation with silver nanoparticles for water filter can function well without any power supply since it only working using gravitational force. Thus, it is suitable for use during emergency situations.

Developing countries would demand for cost effective, affordable and appropriate ways of having clean drinking water supply, especially during emergency situation. This point-of-use water treatment using low-cost material of cellulose filter paper incorporating with silver nanoparticles can be one of the answers. The study will benefit in such way of testing the performance of filter paper coated with silver nanoparticles as antibacterial water filter which at most will provide an easy access for clean drinking water supply during emergency situations.

1.4 Conceptual Framework

The conceptual framework (Figure 1.1) for this study was constructed based on the area of study interest. Generally, the study concept focus on laboratory test for

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Escherichia coli removal (dependent variable) from the application of cellulose filter paper coated with silver nanoparticles (independent variables).

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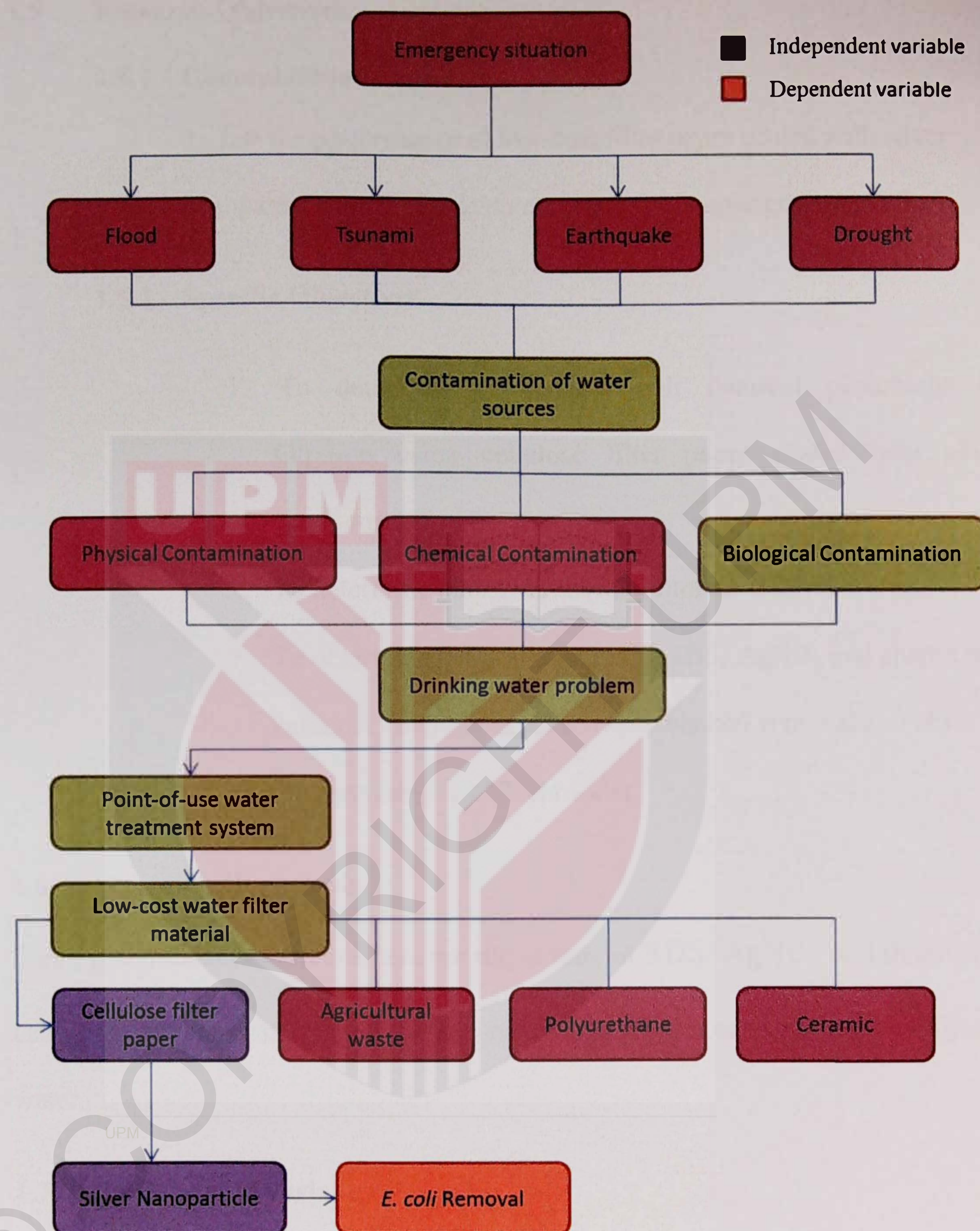

Figure 1.1: Conceptual framework model

1.5 Research Objectives

1.5.1 General Objective

To test the performance of low-cost filter paper coated with silver nanoparticle for *Escherichia coli* removal in emergency situation.

1.5.2 Specific Objectives

1. To determine Escherichia coli removal percentage by filtration using cellulose filter paper coated with silver nanoparticles.
2. To determine the silver concentration in effluent water.
3. To determine the best molar ratio SDS/ $AgNO_3$ and shapes of cellulose filter paper for *Escherichia coli* removal and silver concentration in effluent water.

1.6 Research Hypothesis

There is significant difference between molar ratio of SDS/ $AgNO_3$ and shape of cellulose filter paper in *Escherichia coli* removal and silver concentration in effluent water.

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1.7 Definition of variables

1.7.1 Conceptual definition

1.7.1.1 *Escherichia coli (E. coli)*

Escherichia coli (*E. coli*) are bacterium that found in the gut of humans and animals. Some *E. coli* strains can cause disease. For example, enterotoxigenic strains produce a toxin in the gut, resulting typically in diarrhoea, but can also lead to more serious disease (Stein, 2004).

1.7.1.2 Silver nanoparticles

Silver nanoparticles are crystalline particles of silver metal with at least one dimension between 1 to 100 nm. The morphology of silver nanoparticles varies in shape such as spherical, prism, cubic, or rod in shape (Dankovich, De Moura, Mattoso, & Zucolotto, 2012).

1.7.2 Operational definition

1.7.2.1 *Escherichia coli (E. coli)*

Escherichia coli (E. coli) are type of bacteria species that detected in environmental waters which are more associated with the faecal contamination from people and warm blooded animals (EPA, 2011).

1.7.2.2 Silver in effluent

Silver in the effluent after filtration to be considered to evaluate the impact of the cellulose filter paper on drinking-water quality (Praveena et al., 2016).

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CHAPTER 2

LITERATURE REVIEW

2.1 Drinking water problem during emergency situation

During emergency situation, people will suffer from a lot of difficulties. Safe drinking water is one of the vital aspects to save lives during emergency situation (Praveena et al., 2016). Thus, it is crucial to prioritize the supply of clean potable drinking water during emergency. Unclean water supplies, as suggested by Miles et al. (2009) could pose public to a great danger of waterborne disease outbreak. Butler et al. (2013) mentioned that there are two conventional modes of supplying clean water during emergencies; either water supplied from a packaged treatment plant or trucked water from such a plant and the use of point-of-use water treatment systems. Unfortunately, trucked water is not reliable way since it is known to be expensive and agreed to be difficult in term of transportation and logistics (Praveena et al., 2016). The use of point-of-use water treatment systems is most preferable way to supply clean drinking water during emergency situation. Dankovich et al. (2011) and Clasen et al. (2006) mention that point-of-use water treatment system has been proved effective, economical, energy efficient, and easy to use. Although water treatment have shown a great improvement especially for point-of-use in term of water quality and reducing the risk of waterborne disease outbreak during emergencies, some other limitation need to be overcome including the supply during emergencies in large geographical areas or countries (Doocy et al., 2006).

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According to Brennan & Rimba, (2005), tsunami occurrence December 2004 in Aceh Province, Indonesia, demonstrate that 100% of the survivors drank from unprotected wells and that 85% of residents reported to experience diarrhea after a rapid health assessment conducted in the town of Calang. Meanwhile in Muzaffarabad, Pakistan, an outbreak of acute watery diarrhea occurred in an unplanned, poorly equipped camp whereas 1,800 persons involved after the 2005 earthquake. The outbreak involved more than 750 cases, mostly in adults, and was controlled after adequate water and sanitation facilities were provided (WHO, 2005). Thus, the need for rapid access for potable clean drinking water supply must be highlighted to overcome the problem of having limited clean drinking water problems during emergency situations.

2.2 Incorporation of silver nanoparticles in low-cost materials

According to Praveena & Aris, (2015), low-cost material such as ceramic, polyurethane, agricultural wasted and fibre coated with silver nanoparticles had been used widely as antibacterial filters. The review shows a very significant result in removal of *Escherichia coli* ranging from 97.8% -100% (Jain and Pradeep, 2005; He et al., 2013; Rayner et al., 2013). Although these materials have been proven to have a significant results, some limitation still exist on each and particular materials. Rayner et al. (2013) suggest that even though ceramic filters coated with silver nanoparticles can remove 97.8% to 100% of *Escherichia coli* , effluent filtered shows that the concentration level of silver remained in water exceeds the drinking-water quality standard. Besides, preparation of silver nanoparticles-coated ceramic involve with combustion and long preparation with low flow rate makes this technology unsuitable to be used during emergency situation. Activated carbon

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prepared from rice husk ash contains nearly 95% of silica mass is a reproducible resource for silver nanoparticle incorporation. Unfortunately, the preparation of rice husks also required for combustion method before being coated with silver nanoparticles (He et al., 2013). Due to complex procedure of polyelectrolyte multilayer modification, the applications of polyurethane foam for emergency situation is considered to be not practicable.

Praveena et al., (2016) have done a comparison of difference materials used as antibacterial water filter. Table 2 shows the comparison from previous study of difference materials coated with silver nanoparticle used as antibacterial water filters. Dankovich and Gray (2011) study on blotting paper incorporating with silver nanoparticles state that the percentage of *Escherichia coli* removal is similar to cellulose filter paper. However, the silver concentration in effluent water observed is higher compared to cellulose filter paper. Ceramic studies on incorporating silver nanoparticles to be used as water filter have been conducted by researchers and found that the percentage of *Escherichia coli* removal efficiency is 92% to 100% with continuous operation (Oyanedel-Craver and Smith, 2008; Kallman et al., 2011). Ceramic filters are mainly used in industrial design and for manufacturing purposes using high purity of materials. Because of that, ceramic filters have found to be expensive and not suitable to be used during emergency situation.

According to Jain and Pradeep (2005), polyurethane foam incorporating with silver nanoparticles shows very significant removal efficiencies when there are no colonies of bacteria found in polyurethane foam after filtration process. Furthermore, silver concentration in effluent of filtrate water using polyurethane foam result in zero reading. However, Jain and Pradeep (2005) mention that the process of preparation polyurethane foams involved with repeated washing and air drying.

Thus, polyurethane foams are not suitable to be used during emergencies situations due to the longer preparation process and more costly.

Cellulose filter paper coated with silver nanoparticles shows a significant *Escherichia coli* removal efficiency compared to other materials with percentage of 99% to 100% of removal. Praveena et al., (2016) state that the silver concentration in effluent water filtrated using cellulose filter paper coated with silver nanoparticles results in acceptable concentration (below 0.1 mg/L) which is below the WHO (2004) and USEPA (2011) drinking-water standards. In contrast, the preparation of silver nanoparticle-coated filter paper is easy and short method. Thus, Praveena et al., (2016) suggest the application of silver nanoparticle-coated cellulose filter paper to be used as an antibacterial water filter during emergency situations.

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Table 2.1: Comparison from previous studies of difference materials coated with silver nanoparticle used as antibacterial water filters

Type of materials	*Escherichia coli* removal efficiency (%)	Silver concentration in effluent water (mg/L)	Reference
Cellulose filter paper	99-100	0.02-0.03	Praveena et al. (2016)
Blotting paper	99-100	0.0475	Dankovich and Gray (2011)
Ceramic	92-100	<0.1	Oyanedel-Craver and Smith (2008), Kallman et al. (2011)
Polyurethane foams	100	0	Jain and Pradeep (2005)

2.3 Cellulose

Cellulose is a natural biopolymer and natural fibre which can be found abundantly on earth. It is a non-toxic material which is known to be renewable and biodegradable. According to Peng et al. (2011), cellulose is molecules which have a high degree of functionality. This is due to its chemical characteristic which is carbohydrate polymer and made up of repeating beta D-glucopyranose units and consists of three hydroxyl groups per anhydroglucose unit (AGU). This renewable material has been widely studied, focusing on their biological, chemical, as well as mechanical properties (Peng et al., 2011). Cellulose derivatives has been utilize in several

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industries especially on paper production and printing as well as other industries such as food, cosmetic, oil well drilling, textile, pharmaceutical, etc. and domestic life (Varshney & Naithani, 2011). Moreover, this natural fibre such as bacterial cellulose, filter paper and cotton fabric, have been used widely around the globe as antibacterial water filters (Praveena et al., 2015).

Pinto et al. (2013) suggested that cellulosic material has an excellent effect on the antibacterial efficiency of the nanocomposites. Besides, adsorbent of cellulose filter paper provide an acceptable rapid flow as it depend only on gravity force without using any pressure or suction mechanism (Dankovich and Gray, 2011). Furthermore, the porosity of cellulose filter paper will allow bacteria such as *Escherichia coli* to come into contact with the coated silver nanoparticles in the cellulose filter paper (Praveena et al., 2015).

2.4 Silver nanoparticles

According to Dankovich et al., (2012), silver nanoparticles are crystalline particles of silver metal with at least one dimension between 1 to 100 nm. The morphology of silver nanoparticles varies in shape such as spherical, prism, cubic, or rod in shape. Furthermore, silver nanoparticles are much easier to incorporate into matrix materials, such as paper which contained cellulose, polymers and ceramics. Other than that, due to the nanometer-sized diameters of the silver nanoparticles, the surface area is much greater than other bulk metal, results in more bio-active.

A study conducted by Sondi & Salopek-sondi, (2004) revealed that silver nanoparticles give damage to the cell of the *Escherichia coli*. This is confirmed when the formation of "pits" in the cell wall of the bacteria being observed. The present of

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silver ions from silver nanoparticles in cellulose filter paper attached to the thiol group of the bacteria membrane protein and thus will cause the damage of the membrane (Li et al., 2008; Kim et al., 2011).

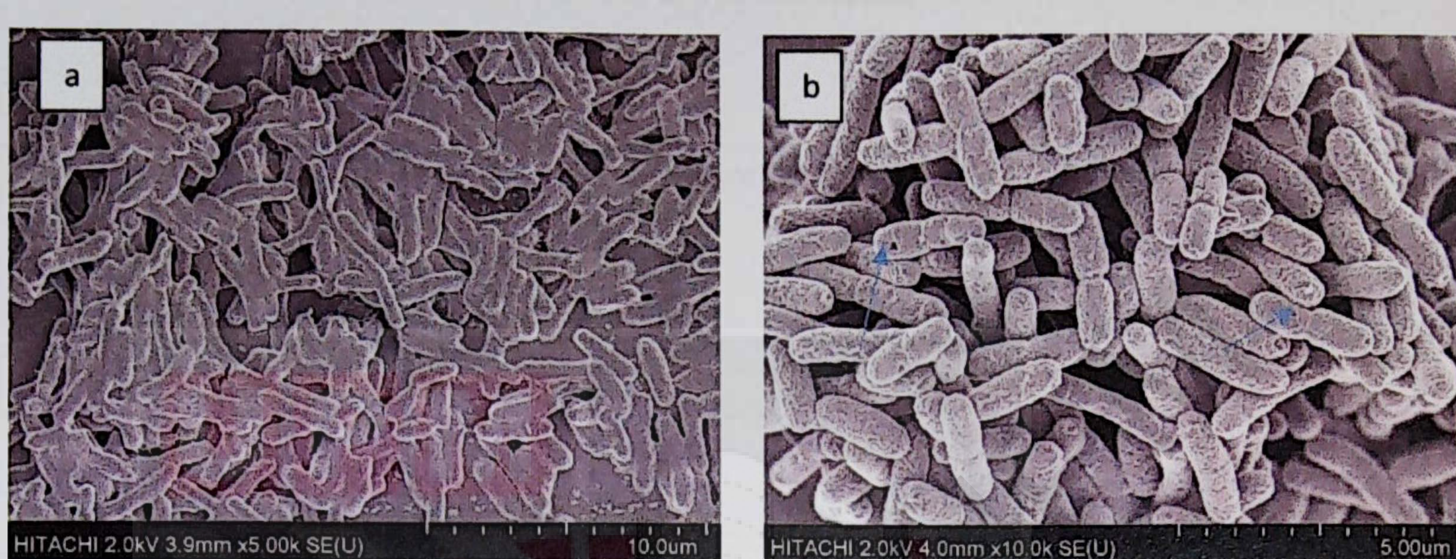

Figure 2.1: SEM images of *E. coli* (a) before filtration without silver contact (b) after filtration with contact of silver nanoparticle (surface cell of *E. coli*).

According to Praveena et al., (2016), for the *E. coli* before filtration that has no silver contact showed the undamaged *E. coli* cell surface as in Figure 2.1(a). After filtration, the morphological changes in *E. coli* observed using a scanning electron microscope as showed in Figure 2.1(b) demonstrated that obvious pits can be found on the *E. coli* cell surface. The formation of these pits can increase cell permeability and lead to cytoplasmic cell leakage.

2.5 Example of water filter product designed for emergency situation

Recently, there are a number of water filter products that have been invented especially for emergency preparedness. Invention of water filter product to be used during emergency situation at most can decrease the problems of limited clean drinking water problem. Table 1 consists of three examples of difference water filter products available in current market.

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Table 2.2: Examples of difference water filter products available in current market for emergency situation use.

No.	Water filter product	Specifications
1.	Aquamira Frontier Water Filter	• Emergency water filter System for especially for emergency situation • Effective on protozoan cysts. • Economical - 1 System filters up to 20 gal. (75L) of water • Lightweight and compact emergency water filter system; fits into a pocket. • Removes greater than 99.9% of Cryptosporidium and Giardia providing safe drinking water. • Can be drunk directly from any water source. • One time use; filters up to 30 gallons of water. • Activated coconut shell carbon filter with Miraguard Technology suppresses microbial agent such as bacteria, algae, fungus, molds and mildew within the filter.

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2.	Katadyn Combi Filter	• Katadyn's proven 0.2 micron ceramic filter has been combined with an activated carbon filter, creating a system that eliminates not only bacteria and microorganisms, but also noxious chemicals & most bad taste. • A high performance, easy-to- use water treatment plant that can be taken anywhere. • Up to 13,000 gallon capacity with cleanable silver impregnated ceramic element. • Field cleanable.
3.	Katadyn TRK Drip Ceradyn (Ceramic) Filter (in Gravity Container)	• Exclusive silver impregnated ceramic elements (3) last up to 39,000 gallons. • Field-cleanable 0.2 micron ceramic depth filter. • Output approx. 1 gallon (4 liters) / hour. Weight = 7.3 lbs. Height = 18 inches. • Comes with 3 cleanable filter elements, measuring gauge and cleaning pad. • Works on gravity pressure from upper compartment; no hook-up required. • Raw water is filled on top with up to 2 1/2 gallons of untreated water and slowly drips through the three ceramic elements into the bottom container.

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Aquamira Frontier Water Filter has been invented specifically for emergency situation use. It is effective to be used on protozoan cysts. Besides, this product specification stated that it is functioning well on suppresses microbial agent such as bacteria etc. However, some limitations that come around are the availability of the product. This Aquamira Frontier Water Filter is not local made product. Moreover, high cost per unit of the product also one of the limitations when it take into consideration of having low-cost products as for emergency situations. Meanwhile, for both Katadyn Combi Filter and Katadyn TRK Drip Ceradyn Filter, even though it has high efficiency of water filtration, these two products also not a local made products. Furthermore, ceramic which have been used as filter element for both of these products will not be functioning well for removal of bacteria and viruses.

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CHAPTER 3

METHODOLOGY

3.1 Study Design

The study was an experimental study design. This study design offer the applicable method for researchers to be able to investigate causality due to the high degree of control (Beaumont, 2009).

3.2 Study Methodology

There were seven stages involved in this study methodology. The steps began with material preparation and followed with silver nanoparticle-coated preparation, bactericidal testing, filtration process, silver-in-effluent analysis and *Escherichia coli* removal effectiveness assessment.

3.2.1 Material Preparation

For material preparation, Whatman filter paper from high-quality cotton liner with 98% of alpha cellulose content was used. High percentage of alpha cellulose is the indication for high amount unmodified cellulose insoluble in sodium hydroxide solution and the pure form of cellulose (Qi et al., 2014). In addition, according to

Pinto et al. (2013), alpha cellulose has highest degree of polymerisation and form most stable material. The total surface area of the cellulose filter paper used were greater than previous study conducted by Praveena et al. (2016).

Cellulose filter paper coated with silver nanoparticle was prepared into two difference shape; 1) rectangular shape and 2) round shape. Each shape of cellulose filter paper coated with silver nanoparticle was placed horizontally on difference design. The detailed of the total surface area for the cellulose filter paper described in table 3.1.

Table 3.1: The total surface areas for the cellulose filter paper design.

Design	Total surface area (m^3)
Rectangular shape	1.68
Round shape	1.32

3.2.2 Silver nanoparticle-coated paper preparation

For silver nanoparticle impregnation into cellulose filter paper, chemical reduction method was applied. Silver nitrate (0.001 M) was used as precursor for silver nanoparticles while sodium borohydride (0.002 M) was used as reduction reagent. According to Praveena et al. (2016) sodium borohydride can induce the most uniform and smallest of nanoparticles among all reducing agents. Meanwhile, sodium dodecyl sulphate (SDS) was applied in this study as stabilizer for the silver nanoparticles. The main purpose of SDS introduced in this study was to prevent the silver nanoparticles from growth and aggregation (Song, Lee, Park, & Lee, 2009). Sodium dodecyl sulphate (SDS) with 6 difference ratios; (0.007 M, 0.024 M, 0.059 M, 0.118 M, 0.177 M and 0.236 M) were used in this study to determine the best

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combination of silver nitrate ($AgNO_3$) with Sodium dodecyl sulphate (SDS) in *Escherichia coli* removal and also silver concentration in effluent. Figure 3.1 explained more on silver nanoparticle-coated paper preparation process flowchart.

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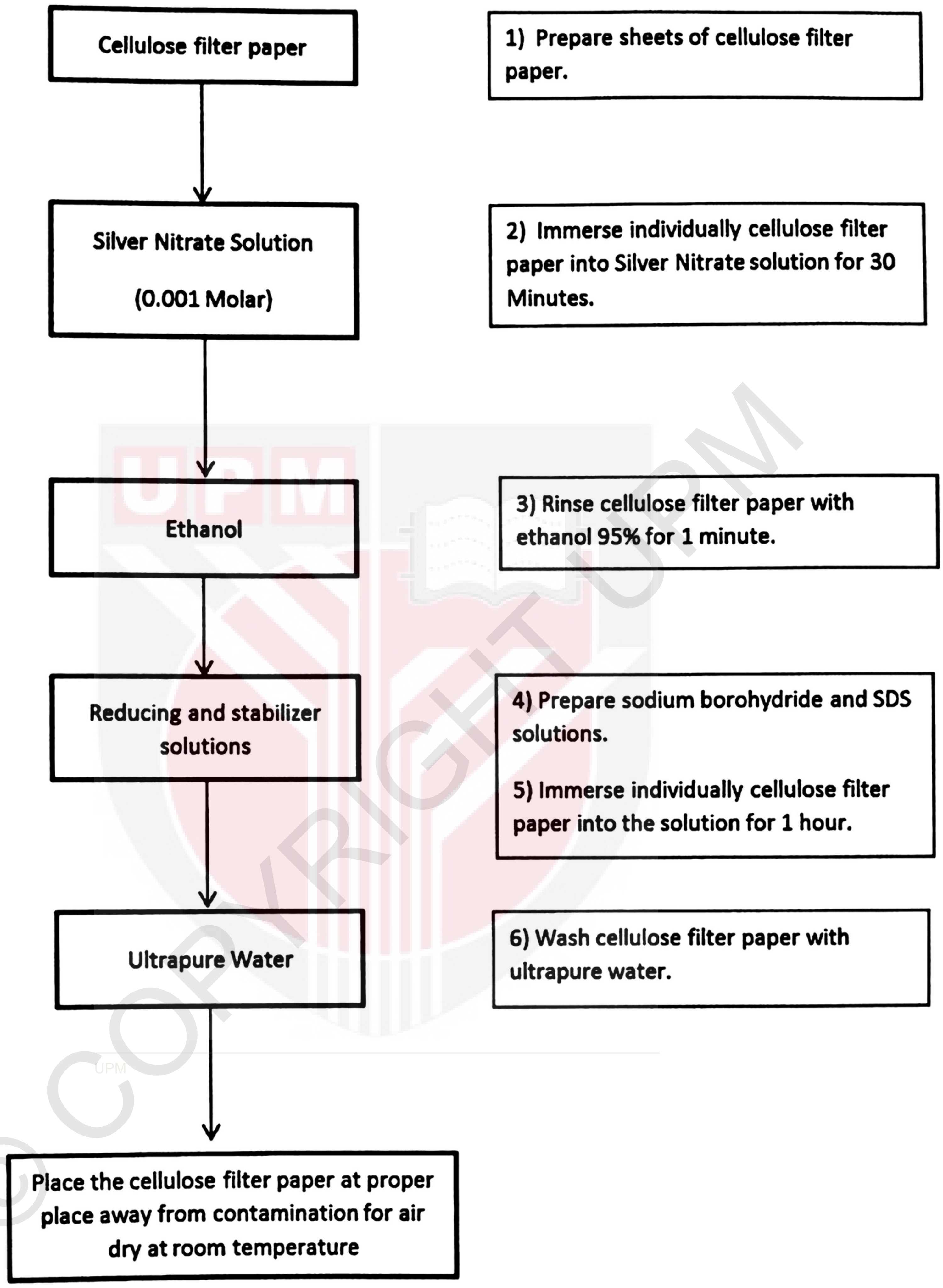

Figure 3.1: Silver nanoparticle-coated paper preparation

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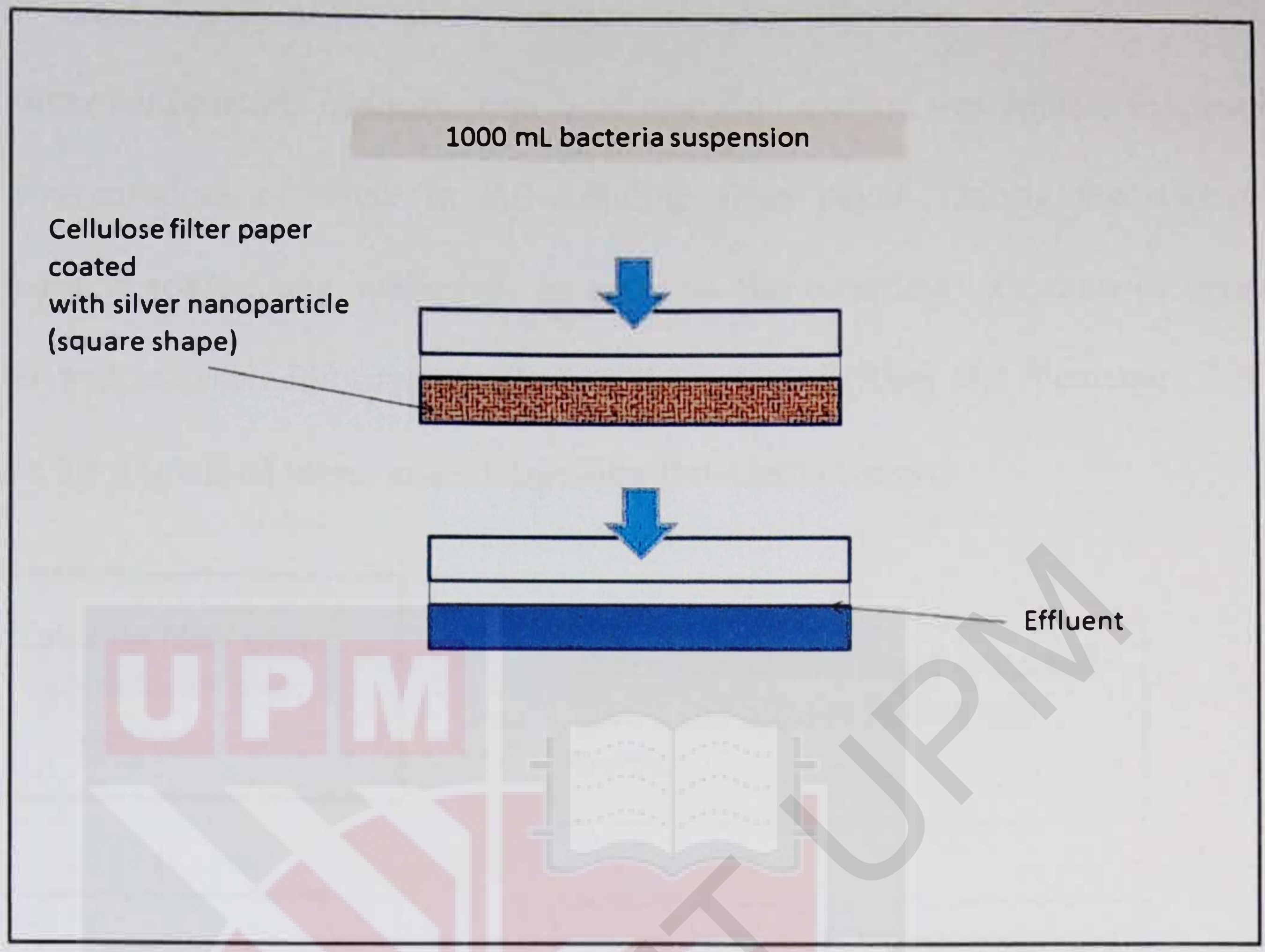

Figure 3.2: Water filtration model (Rectangular shape)

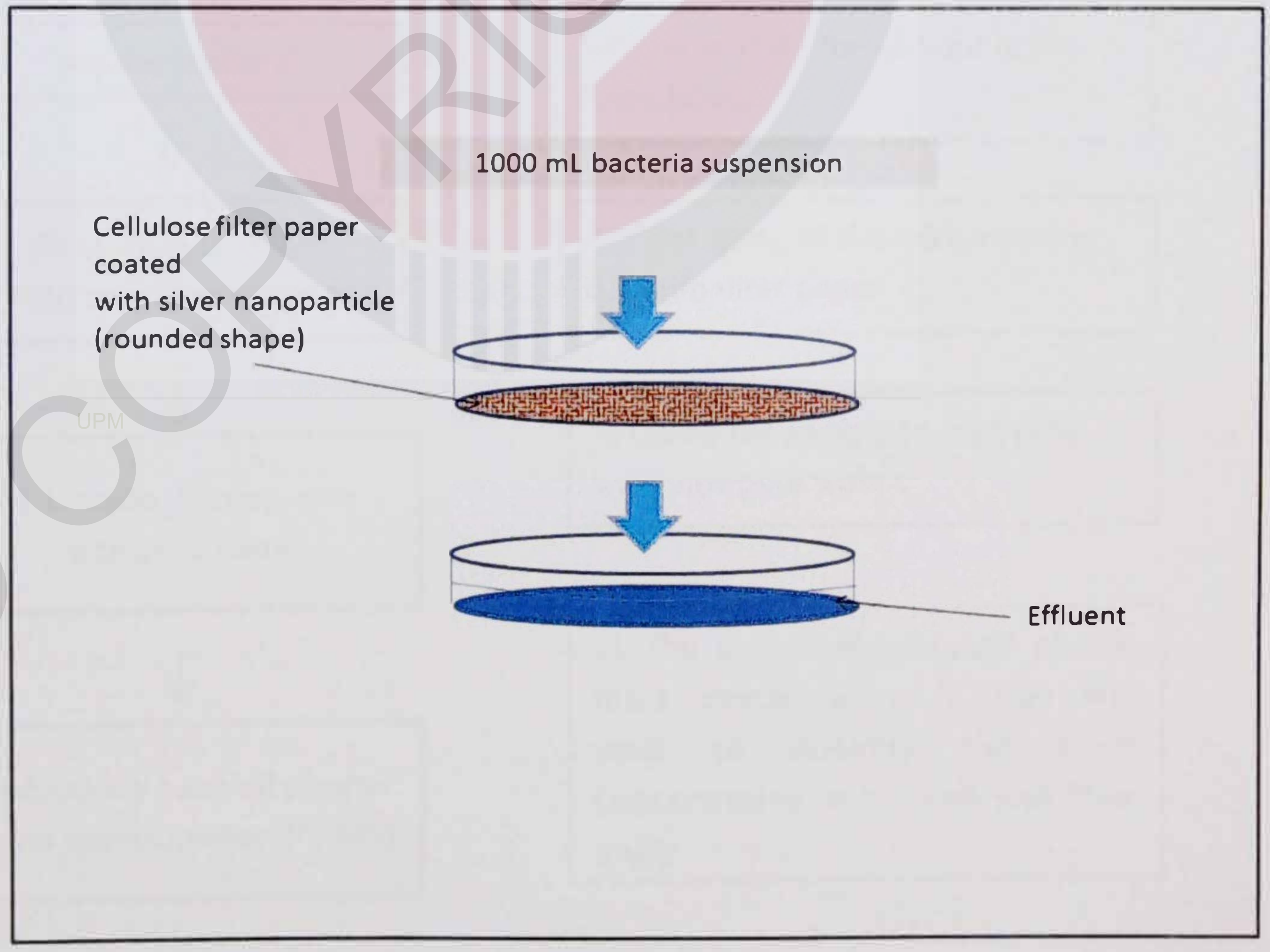

Figure 3.3: Water filtration model (Round shape)

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3.2.3 Acid digestions for silver nanoparticle quantification

For silver nanoparticle quantification, acid digestion method was applied to quantify the concentration of silver in the cellulose filter paper. During the digestion, hydrogen peroxide was applied as to assist in the complete oxidation of organic matter and release additional metals into the solution (Xing and Yeneman, 1998). Figure 3.4 explained more on acid digestion flowchart process:

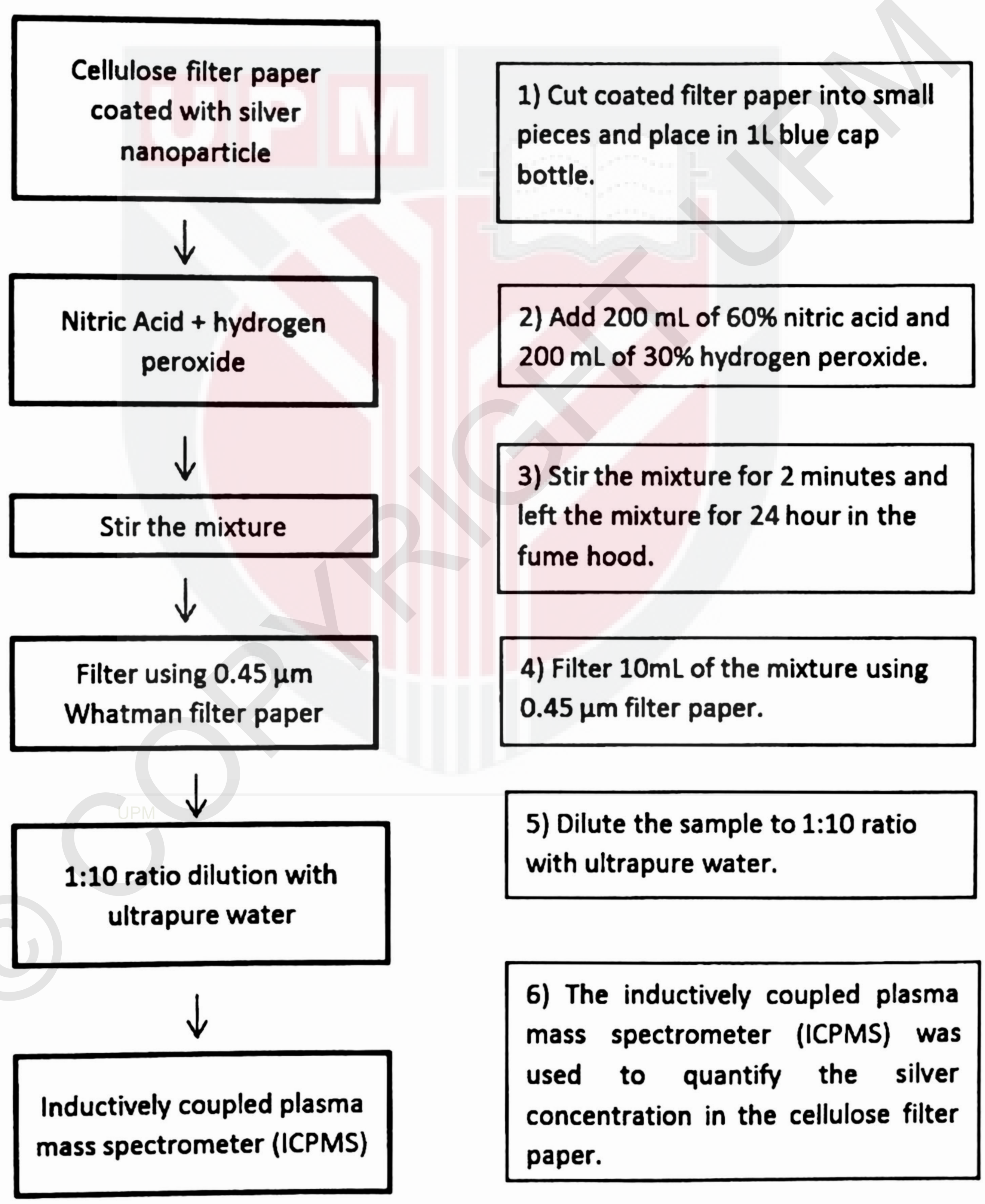

Figure 3.4: Acid digestion process flowchart

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3.2.4 Bactericidal testing

In bactericidal testing, the performance of silver nanoparticle-coated cellulose filter paper to deactivate *Escherichia coli* was tested using a non-pathogenic strain of *Escherichia coli* (ACC 11229). According to Kallman et al, (2013), the organism is a specific indicator for faecal contamination of drinking water. The size and shape of the organism which relatively small (approximately 2.5 μm) make the particular organism ideal for this study.

Sample water containing *Escherichia coli* was prepared to represent the contaminated water. Bacteria were cultured in LB agar plate for overnight before being sub-culture in a broth form. The sub-culture then was incubated in incubator shaker for overnight. Then, bacteria was diluted with autoclave broth and measured to initial absorption of approximately 0.5 by UV Spectrophotometer at 600 nm represented 10^7cfu/mL. Next, saline water were prepared by dissolving 9g of sodium chloride (NaCl) in 1L of ultrapure water and autoclave for 20 minutes at 121 C°. Saline water used to control the rapid growth of bacteria and retained the amount of bacteria required (Sherman et al., 1922). Artificial *Escherichia coli* water sample was prepared by adding one mililiter of *Escherichia coli* suspension of 10^7cfu/mL into one liter of saline water to represent 10^4 cfu/mL. According to Campanhã et al., (1999), a culture with 10^4 cfu/mL was considered to be borderline for the presence of *Escherichia coli.* The removal percentage of *Escherichia coli* was calculated using percentage reduction calculation with following equation;

$$\frac{(E0 - E1)}{E0} X\,100$$

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Where;

E0: The concentration of *Escherichia coli* before filtration,

E1: The concentration of *Escherichia coli* after filtration

3.2.5 Filtration process

In filtration process, sample water containing *Escherichia coli* was used as a model for contaminated water. One liter of water sample containing $1X10^4$ cfu/mL was passed through a sheet of cellulose filter paper coated with silver nanoparticle.

A hundred mililiter of the filtered water was taken to observe the effectiveness of cellulose filter paper coated with silver nanoparticle in removal of *Escherichia coli*. The filtered water was filtered under vacuum through a 0.45 µm Whatman filter paper. The retained *Escherichia coli* on the surface of the filter paper were incubated on m-ColiBlue24 broth at 35° C for 24 hour

3.2.6 Silver-effluent analysis

For silver concentrations in the effluent after filtration process, analysis was done using an Agilent 7500a ICPMS.

3.3 Quality Control

All apparatus used in this study were rinsed with tap water and then soaked in detergent for 20 minutes. The apparatus were then brushed to ensure no impurities remain at the part of apparatus that may contaminate the analysis. The apparatus were then rinsed with distilled water to remove all the remaining detergent and allowed to air dry.

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Centrifuge tube which used was to contain the sample prior to analysis was washed with acid wash overnight and then had been rinsed with distilled water and allowed for air dry. This was done to prevent contamination upon the sample.

During the experiment, 70% of methanol was used to decontaminate of all working surfaces prior to experimentation. In according to Prabha, (2010) handling of toxic chemical was performed in the fume hood.

For glassware and culture media used, autoclaves were done at temperature of 121°C for 15 minutes to sterilization each time prior to be used (Ravikrishna, 2013). For quality control, samples were analysed in duplicates to increase the accuracy and precision. Furthermore, Agilent 7500a ICPMS used in the experiment were calibrated prior to sample analysis to ensure the validity of the data (Chapman & Jackson, 1996).

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CHAPTER 4

RESULTS AND DISCUSSION

4.1 Descriptive statistic for *Escherichia coli* removal, silver concentration in blotting paper and silver concentration in effluent water.

Table 4.1: Descriptive statistic for *Escherichia coli* removal

Ratio $SDS/AgNO_3$	Escherichia coli concentration (cfu/mL)			
	Before filtration		After filtration	
	Round	Rectangular	Round	Rectangular
0.5	$1X10^4$	$1X10^4$	0	0
2	$1X10^4$	$1X10^4$	0	0
5	$1X10^4$	$1X10^4$	0	0
10	$1X10^4$	$1X10^4$	0	0
15	$1X10^4$	$1X10^4$	0	0
20	$1X10^4$	$1X10^4$	0	0

Table 4.2: Descriptive statistic for silver concentration in cellulose filter paper and in effluent water.

Ratio $SDS/AgNO_3$	Silver concentration			
	In cellulose filter paper (mg/m^3)		In effluent water (mg/L)	
	Round	Rectangular	Round	Rectangular
0.5	0.00939	0.00754	0.21960	0.53185
2	0.00981	0.01002	0.48875	1.05485
5	0.00691	0.00952	0.75635	1.54460
10	0.00716	0.02540	0.71320	1.5860
15	0.00740	0.00737	0.82555	0.9314
20	0.01121	0.01160	0.69865	0.9224

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The descriptive statistics for *Escherichia coli* removal, silver concentration in blotting paper and silver in effluent were summarized in table 4.1 and table 4.2 respectively. Table 4.1 consisted of the concentration of *Escherichia coli* before and after filtration and table 4.2 consisted of reading obtained from ICPMS for silver concentration in blotting paper and silver concentration in effluent for both filter paper designs; round and rectangular shape. The artificial water samples with 10^4 cfu/mL of *Escherichia coli* have been reduced in term *Escherichia coli* removal. This have been indicate by the absent of bacteria colony observed after filtration process for all samples.

Table 4.2 demonstrated the result for silver concentration in blotting paper and silver concentration in effluent water. For silver concentration in blotting paper, the highest value in round shape blotting paper was 0.01121 mg/m^3 and the lowest value was 0.00691 mg/m^3. While for silver concentration in rectangular shape filter paper, the highest value of was 0.02540 mg/m^3 and the lowest value was 0.00737 mg/m^3. The silver concentration in cellulose filter paper obtained from acid digestion showed the amount of silver nanoparticle deposited on cellulose filter paper (Dankovich et al., 2012).

For silver concentration in effluent, it was the indication for silver leached form cellulose filter paper into the effluent water (Wan & Karim, 2011). From the result, the highest silver concentration in round shape cellulose filter paper was 0.82555 mg/L for SDS/$AgNO_3$ ratio of 15 and for the lowest silver concentration was 0.21960 mg/L which for SDS/$AgNO_3$ ratio of 0.5. While for rectangular shape cellulose filter paper, the highest value for silver concentration in effluent was

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1.5860 mg/L for $SDS/AgNO_3$ ratio of 10 and the lowest silver concentration was 0.53185 mg/L for $SDS/AgNO_3$ ratio of 0.5.

The retention of silver nanoparticles in the cellulose filter paper was dependable to the method of silver incorporation (Reidy et al., 2013). Hence, the leached of silver into effluent water rely on the incorporation method of silver nanoparticle to cellulose filter paper. As being demonstrated by Song et al., (2009), Chemical reduction method using SDS as stabilizer to synthesis silver nitrate into colloidal silver nanoparticles was result in the nanocrystalline character and well-dispersed state of the silver particles.

The result showed that there were no concentration of silver in effluent water for both shapes and ratios of $SDS/AgNO_3$ comply with the Malaysian Drinking-Water Quality Standards and the guidelines and regulations of the United States Environmental Protection Agency which respectively mentioned that the maximum level of silver ions in drinking water must be less than 0.05 and 0.10 mg/L.

4.2 Removal percentage of *Escherichia coli*

In this study, the action of silver nanoparticles as an antimicrobial agent was observed by incubating *Escherichia coli* taken from effluent on m-ColiBlue24 broth at 35° C for 24 hour. The results revealed that the two tested designs; rounded and rectangular shape of cellulose filter papers coated with silver nanoparticle with six difference ratios of $SDS/AgNO_3$ performed high level removal efficiency for *Escherichia coli.* The results of *Escherichia coli* removal percentage were summarized in table 4.3.

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Table 4.3: *Escherichia coli* removal percentage for round and rectangular shape designs coated filter paper.

Ratio $SDS/AgNO_3$	Concentration of $AgNO_3$ (mol)	*Escherichia coli* removal percentage (%)	
		Round	Rectangular
0.5	0.001	100%	100%
2		100%	100%
5		100%	100%
10		100%	100%
15		100%	100%
20		100%	100%

Table 4.3 demonstrated that the *Escherichia coli* removal percentage for both designs of filter paper with maximum of 100% removal percentage. High removal percentage of *Escherichia coli* was due to interaction between bacterial cells and toxic properties of silver nanoparticle upon the bacteria. *Escherichia coli* were believed to be inactivated by this mechanism (Sondi et al., 2004). According to Praveena et al., (2016), the formation of pits observed on the *Escherichia coli* cell surface after the filtration process. The formation of these pits was an indication for *Escherichia coli* cell damaged and increased of cell permeability in which lead to cytoplasmic cell leakage. There were studies showed that electrostatic attraction between negatively charged bacterial cells and positively charged nanoparticles is crucial for the activity of nanoparticles as bactericidal materials (Hamouda & Baker, 2000). The mechanism of the interaction between the silver nanoparticles and bacteria remained unresolved. However, the interaction of particles with the "building elements" of the bacterial membrane caused structural damage and degradation which end up with cell death (Sondi et al., 2004).

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Another study conducted by Dankovich et al., (2012) also stated that the growth of bacteria after percolation through the silver nanoparticle coated papers was almost completely deactivated. High removal of *Escherichia coli* claimed that the use of cellulose filter paper coated with silver nanoparticle was able to be used as antibacterial water filter.

SDS that had been used as stabilizer in this study with six difference ratios of SDS/$AgNO_3$ ranging from 0.5 to 20 molar ratios were applied during coating of cellulose filter paper with silver nanoparticle by chemical reduction method to find the optimum ratio of SDS in regard for stable formation of silver nanoparticle. SDS was used as stabilizer to prevent the silver nanoparticles in from growth and aggregation which an increasing of SDS ratio also demonstrated a well-dispersed state of silver nanoparticle (Song et al., 2009). Result from this study showed that SDS ratios of 0.5 to 20 were able to help in 100% of *Escherichia coli* removal. This proved that SDS ratios used can help in the stable formation of silver nanoparticle formed on cellulose filter paper.

4.3 The best ratio of SDS/$AgNO_3$ and best shape of filter paper *Escherichia coli* removal.

Table 4.4: Statistical analysis for ratio of SDS/$AgNO_3$ and shape of filter paper with regard for silver in effluent.

		R^2 Value	P value	Mean
SDS/$AgNO_3$ ratio			0.104	-
Shape	Rectangular	0.860	0.013	1.095
	Round			0.617

*General Linear Model (Univariate)

Since the removal percentage of *Escherichia coli* showed a 100% removal, the determination of the best ratio $SDS/AgNO_3$ and the best shape of cellulose filter paper were determined by analysing the effect of the factors on silver concentration in effluent. General linear model (univariate) has been used to test the significant of each factor upon the silver concentration in effluent. Table 4.3 showed there was no significant difference between $SDS/AgNO_3$ ratio and silver concentration in effluent with P value 0.104 ($P>0.05$). However, it was statistically showed that there was significant difference between the shapes of cellulose filter paper and silver concentration in effluent with P value of 0.013 ($P<0.05$). This indicated that there was significant difference between two shapes of cellulose filter paper; round and rectangular in term of silver concentration in effluent water. Round shape filter paper was determined as the best shape for filtration as it showed the lowest mean of silver concentration in effluent water compared to rectangular shape.

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CHAPTER 5

CONCLUSION, STUDY LIMITATION AND RECOMMENDATION

5.1 Conclusion

As conclusion, 100% of the *Escherichia coli* removal percentage by filtration using cellulose filter paper coated with silver nanoparticle have been demonstrated for all six ratios SDS/$AgNO_3$ range from 0.5 to 20 molar ratio in both filter paper shapes (round and rectangular). The concentration of *Escherichia coli* observed after filtration process was zero cfu/mL. In term of silver concentration in effluent, the result demonstrated that the silver concentrations were not complying with the Malaysian Drinking-Water Quality Standards and the guidelines and regulations of the United States Environmental Protection Agency which respectively mentioned that the maximum level of silver ions in drinking water must be less than 0.05 and 0.10 mg/L. The best ratio of SDS/$AgNO_3$ cannot be determine in regard of no significant difference between ratio of SDS/$AgNO_3$ and silver concentration in effluent. The round shape of filter paper was determined as the best shape for filtration when it show the lowest mean of silver concentration compared to rectangular shape.

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5.2 Study limitation

This study was an experimental study design involving 1L of artificial water prepared in laboratory. Thus, field testing using this filter paper depends on several factors such as water turbidity, amount of water can be filtered and type of water. Also, this study did not consider for control on *Escherichia coli* testing and filtration process.

5.3 Recommendation

As recommendation, further study involving field water is needed to investigate the performance of the cellulose filter paper coated with silver nanoparticle in real-word situation. Future study need to take into consideration on several factors such as water turbidity, amount of water can be filtered and type of water to be filtered as to test the performance of coated paper in real-world situation especially in emergency situation and control of sample need to be consider for untreated cellulose filter paper with silver nanoparticle.

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Appendix 1: Table of Results

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A (1): Calculation for bacteria removal effectiveness for rectangular shape filter paper design.

Ratio SDS/Ag NO_3	Concentration $AgNO_3$ (Mol)	Duplication (D)	Number of colony	Bacteria removal effectiveness (%)	Average
0.5	0.001	D1	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
		D2	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
2		D1	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
		D2	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
5		D1	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
		D2	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
10		D1	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
		D2	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
15		D1	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
		D2	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
20		D1	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%
		D2	0	$\frac{10^4-0}{10^4} X\ 100\% = 100\%$	100%

A (2): Calculation for bacteria removal effectiveness for round shape filter paper design

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Ratio SDS/Ag NO_3	Concentration $AgNO_3$ (Mol)	Duplication (D)	Number of colony	Bacteria removal effectiveness (%)	Average
0.5		D1	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
		D2	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
2		D1	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
		D2	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
5		D1	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
		D2	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
10	0.001	D1	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
		D2	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
15		D1	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
		D2	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
20		D1	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%
		D2	0	$\frac{10^4 - 0}{10^4} X\,100\% = 100\%$	100%