POLY (VINYL ALCOHOL) (PVA) NANOFIBERS EMBEDDED WITH SILVER NANOPARTICLES FOR ANTIBACTERIAL STUDIES-BY ELECTROSPINNING METHOD

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ABSTRACT

In this research antibacterial study has been done by using the polymer nanofibers made from polyvinyl alcohol (PVA) embedded with silver nanoparticles. The smaller size of polymer, the surface area will increase. Simple preparation steps had been done for this study. PVA was selected as the matrix because of its special properties such as is water soluble and has excellent fiber forming ability, biocompatibility, chemical resistance and biodegradability. It is one of the good stabilizing agents. An electrospinning technique was used for the fabrication of polymer nanofibers. Recently, many researches have been done in the fabrication of nanofibers by using electrospinning method. This technique has been chosen by many researchers because of its special properties which gives nanofibers with dimensions ranging from micrometer to a few nanometers. SEM and UV-visible spectroscopy analysis has been done for the characterization of morphology of nanofibers. *E.Coli* has been used as gram negative while *S.Aureus* has been used as gram positive. In conclusion, we state that this polymer nanofibers act as a very good antimicrobial, environmental friendly and also known as “green” nanofibers. In antibacterial studies, when the fibers were heated at higher temperatures, it did not show any antibacterial activity. When it was heated at low temperature, it showed excellent antibacterial activity.
Dalam kajian ini, kajian antibakteria telah dilakukan dengan menggunakan nanofiber polimer yang dibuat daripada polyvinyl alkohol (PVA) yang tertanam dengan partikel perak. Saiz yang lebih kecil polimer, kawasan permukaan akan meningkat. Langkah-langkah persediaan yang ringkas telah dilakukan untuk kajian ini. PVA telah dipilih sebagai matriks kerana sifat-sifat khas seperti larut dalam air dan mempunyai fiber yang sangat baik membentuk keupayaan, Biocompatibility, kimia rintangan dan biodegradability. Ia merupakan salah satu agen penstabilan yang baik. Seseorang teknik electrospinning yang telah digunakan untuk fabrikasi nanofibers polimer. Baru-baru ini, banyak penyelidikan yang telah dilakukan dalam fabrikasi nanofibers dengan menggunakan kaedah electrospinning. Teknik ini telah dipilih oleh ramai penyelidik kerana ia sifat-sifat istimewa yang memberikan nanofibers dengan dimensi yang terdiri dari mikrometer untuk nanometers.SEM beberapa dan analisis spektroskopi UV yang dapat dilihat oleh telah dilakukan bagi pencirian morfologi nanofibers. E.Coli telah digunakan sebagai gram negatif manakala S.Aureus telah digunakan sebagai gram positif. Kesimpulannya, kita menyatakan bahawa mi polimer nanofibers bertindak sebagai antimikrobial yang sangat baik, mesra alam dan juga dikenali sebagai "hijau" nanofibers. Dalam kajian antibakteria, apabila gentian telah dipanaskan pada suhu yang lebih tinggi, ia tidak menunjukkan apa-apa aktiviti antibakteria. Apabila ia telah dipanaskan pada suhu yang rendah, ia menunjukkan aktiviti anti-bakteria yang sangat baik.
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<tbody>
<tr>
<td>μ</td>
<td>Micro</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic Force Microscope</td>
</tr>
<tr>
<td>Ag</td>
<td>Silver</td>
</tr>
<tr>
<td>Ag+</td>
<td>Silver Ion</td>
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<tr>
<td>AgNO₃</td>
<td>Silver Nitrate</td>
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<td>AgNP</td>
<td>Silver Nanoparticles</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
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<tr>
<td>EDS</td>
<td>Energy-Dispersive X-Ray Spectroscopy</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>h</td>
<td>Surface Plasmon Resonance</td>
</tr>
<tr>
<td>ml</td>
<td>Milliliter</td>
</tr>
<tr>
<td>Mbc</td>
<td>Minimum Bactericidal Concentration</td>
</tr>
<tr>
<td>Mic</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>mw</td>
<td>Molecular Weight</td>
</tr>
<tr>
<td>PAN</td>
<td>Poly(Acrylonitrile)</td>
</tr>
<tr>
<td>PU</td>
<td>Polyurethane</td>
</tr>
<tr>
<td>PVA</td>
<td>Polyvinyl Alcohol</td>
</tr>
<tr>
<td>PVP</td>
<td>Poly(N-vinylpyrrolidone)</td>
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<td>S. Aureus</td>
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<td>Surface Plasmon Resonance</td>
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<td>Transmission Electron Microscope</td>
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<td>Ultraviolet Visible</td>
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CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Metal nanostructures are of special rich due to their amazing properties in comparison to the bulk metal and are widely used in magnetic, optical receptors, sensing devices, electronic and catalytic devices (Rotello, V.M. 2004; Hu, J et al., 1999; Cui, Y. et al., 2001; Rai, M. et al., 2009 and El-Saved, M.A. 2001). Due to its extremely smaller size, large surface area and shape-dependent optical properties, silver nanoparticles are used as antibacterial (Cao, G. 2004 and Vigo, T.L. 2001). In biomedical field in term of curing and prevention disease, thousand antimicrobial agents have been developed (Lu, L. et al., 2005; Krishnan, S. et al., 2006; Kurt, P. et al., 2007; Fuchs, A. 2006; Rosemary, M.J. et al., 2006 and Dias, H.V.R. et al., 2006). The greatest actions as biocidal ability and non-toxicity to human cells are the main reasons for silver ions and silver nanoparticles to become excellent antimicrobial agents (Balogh, L. et al., 2001; Ramstedt, M. et al., 2007; Sambhv, V. et al., 2006; Shi, Z. et al., 2004 and Wadhera, M. et al., 2005).
Decades ago, silver and silver salts have been used as antimicrobial agents as curing and preventive agents for human health. During 8th century, silver had been first recorded in medical area such as in blood purifier (Moyer, C.A 1965). Today, silver had been use widely in medical field and act as a treatment for infection in burn (Moyer, C.A et al., 1965; Klasen, H.J. 2000 and Silver, S. et al., 2006). For many cases like infection in burns, traumatic wounds and diabetic ulcer, silver is more recently had been used as biocide (Darouiche, R.O. 1999 and Bolender, M.E. et al., 2006). Recently, many researches had been done using some kind of special microorganisms as possible eco-friendly nanofactories for the synthesis of metallic nanoparticles, (Ahmad, A. et al., 2002) such as cadmium sulfide, (Mukherjee, P. et al., 2002) gold, (Ahmad, A. et al., 2003) and silver (Guingab, J.D. et al., 2007). During the formation of silver nanoparticles to obtain the desired properties, varies method had been done and the most selected method is by reduction the silver salt by using suitable reducing agents such as sodium borohydride (Zhou, G. et al., 2006), sodium citrate (Germain, V. et al., 2005), hydrazine (Sun, Y. et al., 2002), polyol (Luo, C. et al., 2005), etc. In this studies, polyvinyl alcohol has been choose as reducing agents because it is good for environmental.

Latest research show that many synthetic and natural polymer such as poly(ethylene glycol) (PEG) (Xiong, Y. et al., 2006), poly-(N-vinyl-2-pyrrolidone) (PVP) (Vigneshwaran, N. et al., 2007), starch (Huang, H. et al., 2004) heparin, and chitosan (Dai, J. et al., 2002) used as reducing agents for the synthesis of silver and gold nanoparticles. In this study, thin film phases had been choose for antibacterial studies. Research before have been able to create Ag nanoparticles containing electrocatalytically active and antibacterial films of polyethyleneimine–metal complex/poly (acrylic acid). These results show that both the films with Ag⁺ ions and those have nanoparticles work as antibacterial agents. According to them, nanoparticles in films are preferred to reduce the harmful diffusion of Ag⁺ ions into the body (Jaidev, L.R. et al., 2010).
Various method had been used for characterization the nanoparticles such as Ultraviolet spectroscopy analysis, thermal electron microscope analysis and X-ray diffraction analysis (Huang, Z.M. et al., 2003). Spin coating is used for the formation of thin film. By using electrospinning, various type of polymer had been converted into fibers (Li, X. et al., 2004). Some research has been done using hydroxyl cellulose as a stearic stabilizer such as in the synthesis of nanocrystalline ceramic oxide powders (Shukla, S. et al., 2002&2003).

1.2 PROBLEM STATEMENT

Most of the research in nanoparticles involves toxic solvents which are harmful to the environment. In this study, the research approach no toxic solvent, green technology and environmental friendly.

1.3 RESEARCH OBJECTIVES

The main objectives of this research are:

a) To study the efficiency of polymer nanofibers of PVA/Silver nanoparticles in antibacterial studies.

b) To obtain the optimum concentration of PVA and silver nitrate in killing the bacteria.

c) To identify the effect of heating the polymer nanofibers in antibacterial studies.
1.4 SCOPE OF STUDY

In this research, the effectiveness of silver nanoparticles (Ag NP) using PVA solution in antibacterial studies have been focused. The optimum concentration of silver nanoparticles in curing is focused. These are the things that are very important because during the experimental work, the exact amount of concentration used have to be recorded to show the effectiveness. Other than that, we want to identify the effect of heating towards polymer nanofibers in the antibacterial studies.

1.5 SIGNIFICANCE OF STUDY

Nanofibers of polyvinyl alcohol embedded with silver nanoparticles for antibacterial studies will give many benefits and advantage in many fields especially in medicine fields. Chemistry has a very strong bond in every single life even though peoples do not notice that. Significance from this study, even though we use a very small amount of curing but the result will be show an amazing curing. Some creative touch in nanoparticles preparation and play with many concentrations will discovered a lot of mystery. This study will show the effectiveness of silver nanoparticles embedded with polyvinyl alcohol in antibacterial studies by using optimum concentration and also the effect of heating towards antibacterial studies.
CHAPTER 2

LITERATURE REVIEW

2.1 OVERVIEW OF NANOPARTICLES

The term ‘nanoparticles’ is used to describe particles with the size in the range of 1 to 100 nm at least in one of the three dimensions. In this range, the physical, chemical and biological properties are change in fundamental ways from the properties corresponding to the bulk material (Revathi, J. et al., 2009). Generally, there are designed with surface modification tailored to meet the need of specific applications that they are to be used for. The large specific surface area of nanoparticles is the origin of a number of their unique applications. High surface areas give strong interactions between nanoparticles and the solid matrix in which they may be incorporated. Nanoparticles can be synthesized by a variety of methods using solid, gas and liquid phase processes. (Ramanathan, N. et al., 2008). Discoveries in the past decade have demonstrated that the electromagnetic, optical and catalytic properties of noble-metal nanoparticles such as gold, silver and platinum, are strongly influenced by shape and size. (Daniel, M.C. et al., 2004).
2.1.1 Silver Nanoparticles (AgNP)

Among all metal, silver is more interesting due to its application and properties. Silver acts as the stabilizer and reducing agent. Silver, and silver-based compounds, is highly antimicrobial thanks to its antiseptic properties to several species of bacteria, including the common kitchen microbe, *E. coli*. Silver nanoparticles interact with the outer membrane of bacteria, causing structural changes that lead to degradation and eventually death of the microbe. Silver nanoparticles are one of the most commonly utilized nanomaterials due to their anti-microbial properties, high electrical conductivity, and unique optical properties. Silver nanoparticles have so many applications like electronic field, catalysis and wound dressing, but the most important one is antibacterial studies (Resham, B. *et al.*, 2008).

Silver has such advantages as broad spectrum antibacterial studies activity, non-toxicity to human cells and long lasting effect (Yunarova, T. *et al.*, 2003). Silver nanoparticles used in this study were prepared by the reduction of silver nitrate and characterized using UV–Visible spectroscopy (UV–Vis) and transmission electron microscopy (TEM). Transmission electron microscopy (TEM) allows to directly image the lattice structure of nanoparticles in the order of a few nanometers (Yamamuro, S. *et al.*, 2002) as well as to obtain diffraction data, amplitudes and phases of nanoparticle structures. Studies by TEM can be used to determine the behavior and self-assembly of nanoparticles under external influences such as magnetic fields (Ahniyaz, A. *et al.*, 2007).

Furthermore, elemental analysis of nanoparticles can be made using energy dispersive X-ray spectroscopy (EDS), and modern transmission electron microscopes are equipped with tools to perform elemental mapping and analysis using incident probe sizes in the order of a few nanometers in diameter (Fadeel, B. *et al.*, 2010). In order to study the conversion of AgNO₃ to Ag in the PVP nanofibres during the heat treatment, UV–visible absorbance spectroscopy was used; specifically, this can be used to track the formation of silver nanostructures of various dimensions, which exhibit surface plasmon
resonance (SPR) bands at different frequencies (Hernandez, E.A. et al., 2005). Silver nanoparticles also can be prepared by a UV-irradiation photo reduction technique (Ershov, B.G. & Henglein, A. 1993).

2.1.2 Nanoeffects of Silver Particles

About 20 - 50,000 silver atoms had been found in silver nanoparticles. The size of atoms usually smaller than 100 nm (Chen, X. et al., 2008). Basically, total surface energy is lower than single crystal because lower energy faces at the expense of an internal strain. For silver structures, more {111} facets with the lowest surface energy and planes surfaces are preferred when the size of particles decreased to nanosize. This figure shows the highest atomic density. The morphology of silver nanoparticles is favored with high atom density facets such as {111}. Silver nanoparticles with this type of facets will interact with bacteria’s thiol group which are contains sulfur hydrogen bond. Elechiguerra et al had done a study and reported that only special size of silver particles can bound to human within the range 1-10 nm. The strongest bacterial activity occurs at basal planes compare to spherical or rod shaped. The direct interaction between silver particles and bacteria only occur at the diameter of about 1-10 nm.

Figure 2.1: Face centered cubic unit cell of silver

Source: Cao, H. and Liu, X. (2010)
2.1.3 **AgNP in Term of Biocompatibility**

Biocompatibility means the ability to coexist with living organism without harming them. In 1996 studied (Ratner *et al.*, 1996), biocompatibility is “the ability of a material to perform with an appropriate host response in a specific application.” Silver is very great and excellent bactericidal metal because it is non-toxic to human and animal cells but highly toxic to bacteria such as *E.coli* and *S. Aureus* (Klueh, U. *et al.*, 2000 and Zhou, G. *et al.*, 1998). This silver nanoparticles act as biocidal agents. (Shan-hui, H. *et al.*, 2010), Silver (Ag) is regarded as one of the noble metals with high biocompatibility. Both nano Ag and nano Au were reported to modify the microphase separation on the surface of H12MDI-based PU and enhance the biostability and biocompatibility in vitro and in vivo, with nano Ag being more effective (Chou, C.W. *et al.*, 2008).

It is believed that the silver nanoparticles having phosphorylcholine groups to enhance their biocompatibility and intracellular uptake and having rhodamine dye on its surface as a fluorescent probe could be a promising biomedical material (Yi-Chang, C. *et al.*, 2007) search approaches green technology and environmental friendly because water-base system is used during the formation of silver nanoparticles. Water is non-toxicity and does not harmful to the environment and human health. Kulshreshtha, S.N (1998), water is widely used in chemical reactions as a solvent or reactant and less commonly as a solute or catalyst. In inorganic reactions, water is a common solvent, dissolving many ionic compounds. In organic reactions, it is not usually used as a reaction solvent, because it does not dissolve the reactants well and is amphoteric (acidic and basic) and nucleophilic.
2.1.4 Characterization of AgNP

UV spectroscopy is used to characterize the AgNP. UV-visible spectroscopy is the measurement of the wavelength and intensity of absorption of near-ultraviolet and visible light by a sample. In metal nano particles such as in silver, the conduction band and valence band lie very close to each other in which electrons move freely. These free electrons give rise to a surface plasmon resonance (SPR) absorption band occurring due to the collective oscillation of electrons of silver nano particles in resonance with the light wave.

![UV-Vis spectrum of as-prepared Ag NPs synthesized by chemical reduction of Ag+ by dextrose](image)

**Figure 2.2:** UV-Vis spectrum of as-prepared Ag NPs synthesized by chemical reduction of Ag+ by dextrose

Source: Ostad, S.N. *et al.* (2010)
2.2 CHARACTERIZATION OF POLYVINYL (ALCOHOL) (PVA)

PVA is a poly hydroxyl polymer, which is water soluble and has excellent fiber forming ability, biocompatibility, chemical resistance and biodegradability (Lin, W.C. et al., 2006 and Krevelen, D.W.V. et al., 1975). It is also known as a very good stabilizer for some metals particles (Longenberger, L. et al., 1995). In present works, PVA have an ability act as nature reducing agents. In chemical synthesis of nanoparticles, polyvinyl alcohol had been used as a stabilizer. Polyvinyl alcohol is a very good water soluble polymer and suitable for nanofibers preparations (Koski, A. et al, 2004).

PVA also have been known as potential biodegradable polymeric materials for environmental application and also for biomedical (Chen et al., 1997). It also exhibits good mechanical properties; chemical resistance, water soluble and highly crystalline (Fussell, G. et al., 1998). Table 2.1 generally shows about the chemical identity and physical properties of PVA.

Table 2.1: General chemical identity and physical properties of polyvinyl alcohol

<table>
<thead>
<tr>
<th>CAS No.</th>
<th>Molecular weight*</th>
<th>Structural formula*</th>
<th>Empirical formula*</th>
<th>Physical appearance</th>
<th>Specific gravity</th>
<th>Solubility</th>
</tr>
</thead>
<tbody>
<tr>
<td>9002-89-5</td>
<td>30,000-200,000</td>
<td>(CH₂CHOH)ₙ⋅(CH₂CHOCOCH₂)ₘ</td>
<td>(C₄H₆O₅)(C₂H₄O)ₙ</td>
<td>Odorless, white to cream-colored granular powder</td>
<td>1.19-1.31</td>
<td>Insoluble in aliphatic and aromatic hydrocarbons, esters, ketones, and oils; water soluble</td>
</tr>
</tbody>
</table>

* Variable based on PVA grade.

Source: DeMerlis, C.C. et al. (2003)
2.2.1 Classification of PVA.

Table 2.2: Classification of PVA

<table>
<thead>
<tr>
<th>Classification of PVA</th>
<th>Structural Formula</th>
</tr>
</thead>
</table>
| Partially hydrolyzed         | \[
\begin{align*}
CH_2 & - CH \quad CH_2 & - CH \\
OH \quad n & & O \quad m \\
C & = O \\
\end{align*}
\] |
| Fully hydrolyzed             | \[
\begin{align*}
CH_2 & - CH \\
OH \quad n &
\end{align*}
\] |

Source: DeMerlis, C.C. et al. (2003)

Table 2.2 shows the classification of PVA. Polymer is one of the excellent materials for metal and semiconductor (Zhang, Z. et al., 2003 and Hussein, I. et al., 2003). By coated the metal with hydrophilic and biocompatible polymer such as polyethylene glycol (PEG), dextran, polyvinyl alcohol (PVA), poly (acrylic acid), poly (lactide-co-glycolide) (PLGA), chitosan, pullulan, and poly (ethyleneamine) (PEI) (Gupta, A.K. et al., 2005 and Harris, J.M. et al., 2001), the circulation times will be longer. PVA has optimum film forming, emulsifying and adhesive properties. Fabrication and characterization of silver-polyvinyl alcohol nanocomposites were already reported by Mbhele, Z.H. et al., 2003.

PVA is very expensive, has low biodegradation rate and poor moisture properties. To reduce cost and enhance the performance, PVA are modified with other polymer such as nanoparticles and starch (Tang, X. et al., 2011). PVA does not exist in the free states. Therefore, PVA cannot be made by polymerization of vinyl alcohol. For
preparation, they used partial or complete hydrolysis of polyvinyl acetate to remove the acetate group. Since 1930’s, PVA is known as biodegradable synthetic polymer. The problem here is the higher cost compared to other polymer such as polypropylene and polyethylene. The application for PVA is very broad. Usually this polymer had been use for water soluble packaging fills, paper adhesives, textiles and paper coating (Chang, J. et al., 2003 and Ibrahim, M. et al., 2010). This is because of the excellent film forming, emulsifying and adhesive properties of PVA itself. High energy cost of evaporating water needs for this polymer because of it water soluble properties. Table 2.3 show the effect of molecular weight and hydrolysis level on the physical properties of PVA.

![Figure 2.3: Effect of molecular weight and hydrolysis level on the physical properties of PVA](source)

**Figure 2.3:** Effect of molecular weight and hydrolysis level on the physical properties of PVA

2.3 CHARACTERIZATION OF POLYMER NANOFIBERS

The principle of electrospinning method is very simple. The fiber form when the electrostatic field stretches the polymer solution into fiber. But, the process is very difficult to control. Usually, the polymer nanofibers can be characterized based on morphology, mechanical, thermal and chemical properties.

2.3.1 Morphology Characterization

The quality of the fibers is typically inconsistent, for example, the fiber deposition may be uneven or the distribution of fiber diameter. Scanning electron microscope and transmission electron microscope is the instrument that can be used to measure the diameter. But, both of these instruments are not too precise compare to atomic force microscope (AFM) (Srinivasan, G. et al., 1995 and Li, W.J. et al., 2002). For this instrument, a very sharp probe moves over the surfaces. The tip geometry in the AFM make the fiber looks larger than the actual (Jaeger, R. et al., 1996).

2.3.2 Mechanical, Chemical and Thermal Characterization

While decreasing the diameter, the tensile strength will increase. The contact area between filler and polymer increase due to the increase in fiber surface area/volume ratio. Therefore, the flexibility of fiber will increase (Paul, D.R. et al., 2008). AFM is also used to measure the mechanical properties of polymer nanofibers. To increase the mechanical properties of polymer nanofibers, inorganic fiber such as glass and carbon fibers and also aromatic organic fibers had been used in previous study.