약학석사학위논문

# 당뇨병성족부궤양의 치료를 위한 고가 은 나노입자의 응용

# Preparation of High Valence Silver Complex Nanoparticles for Diabetic Foot Ulcers Application

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# 국문초록

당뇨병성 족부궤양(DFUs)은 당뇨병 환자의 건강에 심각한 영향을 미치는 당뇨병의 주요 합병증 중 하나다. 상처치유는 DFUs의 치료에 필수적인 과정이다. 병원성 박테리아의 감염과 관련된 염증은 상처치유의 중요한 역동단계 중 하나다. 항생제는 DFUs에 널리 이용되고 있다. 항생제 내성으로 인해, 은이 함유된 항균제가 더 큰 관심을 불러올 것이고 효과적일 것으로 보인다. 이 문제를 해결하기 위해 역마이크로 에멀젼 기술을 이용하여 더 높은 항균작용의 고가 은 나노입자를 설계하고 합성했다. 증식은 상처치유의 또 다른 필수단계다. 상처치유는 수선 및 세포성장을 위한 아미노산에 대한 요구를 증가시킨다. L-phenylalanine은 필수 아미노산 중 하나다. 즉, 인체에서 합성될 수 없고 음식물로 보충해야한다. 본 연구에서는 은 복합체 합성에 리간드로 작용하는 L-phenylalanine을 함유하는 Boc-L-phenylalanine와 propamidine을 결합했다. 따라서 이 화합물은 아미노산 부분을 포함함으로써 증식성 치료능력을 가진다. Silver (II)-Boc-propamidine 나노입자는 모두 분광기술로 측정했다. 우리는 DFUs의 병원균에 대항하는 silver(II)-Boc-propamidine 나노의 항균활성을 조사하였다. 새로 합성된 고가 은

복합체 나노입자가 은나노입자(AgNPs)보다 높은 항균활성을 나타났다. 이 연구는 합성된 silver(II)-Boc-propamidine 나노입자가 보다 우수한 항균활성을 입증한다고 결론지었다. 당뇨병성 족부궤양의 치료를 위한 차세대 치료제로 사용될 수 있을 것이다.

**주 요 어: Propamidine, Silver nanoparticle, 당뇨병족궤양, 항균, 증식**

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## List of Abbreviations

DFUs: Diabetic foot ulcers

AgNPs: Silver nanoparticles

PHMG: Polyhexamethylene guanidine

Boc-L-Phenylalanine: N-(tert-Butoxycarbonyl)-L-phenylalanine

Boc-Propamidine: Boc-L-Phenylalanine-Propamidine

AOT: Dioctyl sulfosuccinate

EDCl: N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide,hydrochloride

HOBT: 1-hydroxybenzotriazole hydrate

DIEA: N,N-Diisopropylethylamine

MS: Mass spectroscopy

NMR: Nuclear Magnetic Resonance

TMS: Tetramethyl silane

IR: Infrared Spectroscopy

UV-Vis: Ultraviolet–visible spectroscopy

TEM: Transmission electron microscope

MICs: Minimum inhibitory concentrations

MBCs: Minimum bactericidal concentrations

MHB: Muller Hinton Broth

MHA: Muller Hinton Agar

DMEM: Dulbecco's modified eagle's medium

DMSO: Dimethyl sulfoxide

# I. Introduction

Diabetes mellitus is a severe chronic disease worldwide[1]. The sharp rise in the prevalence of diabetes leads to an increase in diabetes related complications[2]. Diabetic foot ulcers (DFUs) is one of the main complications of diabetes mellitus, which seriously affects the health of diabetic patients. Up to 15% to 25% of people suffer from DFUs at some point in all patients with diabetes mellitus[3-4]. DFUs has higher risk in amputation and mortality rate. All chronic wounds are infected by microorganisms[5]. Wound healing is a dynamic process containing collagen synthesis, inflammation, proliferation and tissue remodeling four procedures[6].

Patients with diabetic foot ulcers treated with antibiotics are often infected by gram-positive bacteria and gram-negative bacteria[7]. Antimicrobial agents are widely applied for the treatment of wounds in the presence of wound infection. Topical bactericide can affect wound healing, but silver containing antimicrobial agents appear to be safe and effective. Wound healing requires effective antimicrobial therapy as well as re-epithelialization[8].

People have known the antibacterial properties of silver for centuries around the

world[9]. The use of silver as an antibacterial agent decreased after antibiotics were discovered. Due to the emergence of antibiotic resistance, there has recently been renewed interest in the use of silver as an antibacterial agent[10]. Although the antibacterial properties of silver have been known for a long time, we have only recently begun to understand the antibacterial mechanism of silver. The mechanism of action of silver is related with protein deactivation that the silver atoms bind to the sulfhydryl groups in the enzyme, resulting in the inactivation of the enzyme. Another mechanism is that silver ions released by silver entering the cell and associating with DNA, thus causing bacterial cell damage[11].

Silver nanoparticles particle size is less than 100nm, which are especially advantageous as antibacterial agents in the field of wound healing. It can be applied to antibacterial medicine and medical equipment, antibacterial plastic and rubber products. The application of silver nanoparticles to increase the surface to volume ratio has been reported to be successful in improving the bactericidal efficiency. Thus, an increase in the contact area of nanoparticles is able to produce more silver ions capable of interacting with bacteria, thereby impairing them through multiple pathways. For these reasons, silver nanoparticles have received great attention in the field of wound care[12]. Silver has good

antibacterial activity and moisturizing effect on human skin. Silver nanoparticles have great potential for promoting the re-epithelialization of skin wounds as an ideal therapeutic agent for the treatment of chronic wounds. The key issue is how to develop silver nanoparticles to enhance the proliferation and motility of skin cells.

In recent years, it has been demonstrated that high-valence silver showed stronger antibacterial ability than low-valence silver [13]. These findings have prompted us to attempt to synthesize high valence silver complexes nanoparticles with both antibacterial ability and re-epithelialization activity. Silver ions can form complexes with ligands in the oxidation state of +1, +2, + 3. When ligands complexed with silver ions have auxiliary functions, such as acceleration proliferation and viability, the silver complex can be expected to possess a versatile therapeutic activity. We attempted to prepare high valance silver complex nanoparticles with antibacterial and proliferative function to promote wound healing.

Silver sulfadiazine was applied to prevent wound infection especially for burn wound infection. Silver sulfadiazine is the standard drug for prevention and treatment of severe burn infection. In addition to controlling infection, it can also promote healing. Silver

sulfadiazine has a broad spectrum of antimicrobial activity against many gram negative and gram positive bacteria. It has low toxicity, a broad spectrum of activity against a wide range of microbial pathogens. Silver sulfadiazine can decompose to release silver and sulfadiazine slowly when exposed to tissue[14].The development of antimicrobial reagents has achieved remarkable results [15]. In view of the fact that silver sulfadiazine possess the astringency effect of silver and the antibacterial and anti-inflammatory effects of sulfadiazine, we attempt to prepare a novel silver containing bifunctional antibacterial reagent for diabetic foot ulcers application.

Propamidine is one of the antibacterial agents that can inhibit the growth of microorganisms, including bacteria, viruses, fungi as well as protozoans[16]. It is a type of guanidine aromatic compounds for the treatment of ocular infections. Propamidine is an aromatic diamidine compound, which can be used as a chelating ligand. This ligand with silver can form silver complex which have the potential to be used as a new type of antibacterial agent. Propamidine possess conjugate single and double bond systems and filled with p-type delocalized orbitals suitable for the formation of higher valence silver complexes. It has been shown that the guanidine compounds have been used for

antibacterial and antifungal drugs for many years, such as polyhexamethylene guanidine (PHMG), brolene and chlorhexidine. Silver chlorhexidine complex has been prepared successfully to antibacterial field[17]. Therefore we choose propamidine as the ligand of silver complex. In addition, the amine group in propamidine can be conjugated with amino acid by peptide coupling reaction, thus this compound will possess the potential of proliferation.

The final result of the wound healing process is to repair the defective tissue. Coagulation is an essential process for healing wounds. The goal of treatment is to enhance collagen deposition in order to improve the strength and integrity of the wound. The addition of nutrition can promote wound healing. The wound will increase the demand for amino acids for the repair and cell growth. L-Phenylalanine is one of the essential amino acids for the human body, which means that the body needs this component and cannot produce it naturally[18]. N-(tert-Butoxycarbonyl)-L-phenylalanine (Boc-L-Phenylalanine) is a derivative of phenylalanine can be used as amino acid protection monomers for peptide coupling synthesis[19].

In this study, we conjugated propamidine with Boc-L-phenylalanine which contain phenylalanine moiety as a ligand. Then we synthesized high-valence silver complex

nanoparticles by incorporating Boc-L-phenylalanine-propamidine (Boc-propamidine) moiety using reverse microemulsion technique. The synthesized Ag(II)Pro-Boc nanoparticles possess higher antibacterial activities against the tested gram positive and negative strains responsible for diabetic foot ulcers. Besides, this compound also has the potential proliferative therapeutic ability due to containing amino acid moiety. And it may serve as next generation therapeutic agent for the diabetic wound care.

# II. Experiment Section

## 1. Materials and Methods

Silver nitrate, triethylamine, sodium persulfate, dioctyl sulfosuccinate (AOT), and heptanes used for the synthesis of nanoparticles and N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide, hydrochloride (EDC.HCl), 1-hydroxybenzotriazole hydrate (HOBT), N, N-diisopropylethylamine and hydrochloric acid used for the synthesis of Boc-L-phenylalanine were purchased from Sigma-Aldrich, South Korea. The propamidine was purchased from Stru Chem CO., LTD, China. We bought sodium chloride and aluminum oxide, basic from Alfa Aesar, South Korea.

IR spectrum was obtained from the JASCO FT/IR-4200 spectrometer. Transmission electron microscope (TEM) was recorded in JEM 3010 and JEM 1010, Jeol, Tokyo. The UV-Vis absorption spectrum was recorded in a Thermo Scientific Evolution 60 instrument.

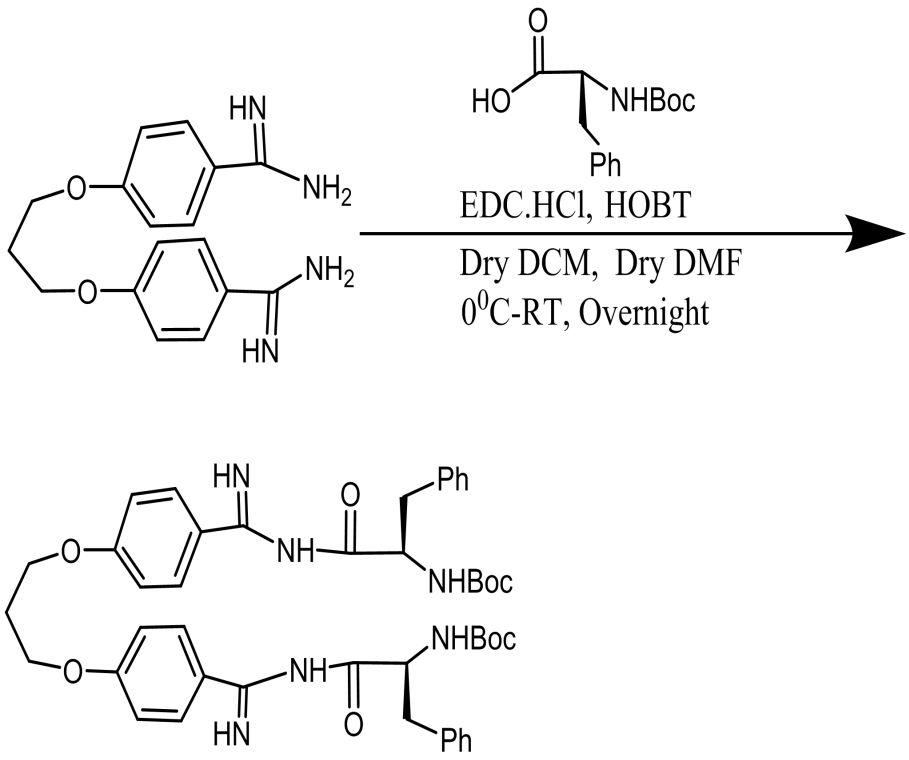


**Figure1.** Preparation Scheme for the Synthesis of Boc-Propamidine

## 2. Synthesis of Boc-L-Phenylalanine-Propamidine

The synthetic scheme for the preparation of Boc-propamidine is shown in Figure 1. The synthesis of Boc-L-phenylalanine-propamidine was performed by amine to amide peptide coupling reaction[20]. We choose EDCI and HOBT as coupling reagent. The synthesis of ligand was performed in dichloromethane:dimethylformamide (2:1) solvent system. 0.8mmol Boc-L-Phenylalanine, EDC.HCl and HOBT was dissolved in 15ml dichlormethane. The reaction mixture was stirred for 1 h at 0 ° C under nitrogen environment. Then add the solution of 0.32mmol propamidine dissolved in 7.5ml dimethylformamide to the mixture and use 0.5 ml N,N-Diisopropylethylamine (DIEA) to activate reaction. The reaction was stirred at room temperature for 16h. After the reaction, workup and purify the compounds by basic alumina column. The product was dried and analyzed by Mass spectroscopy, $^{1}$H NMR and FT-IR .

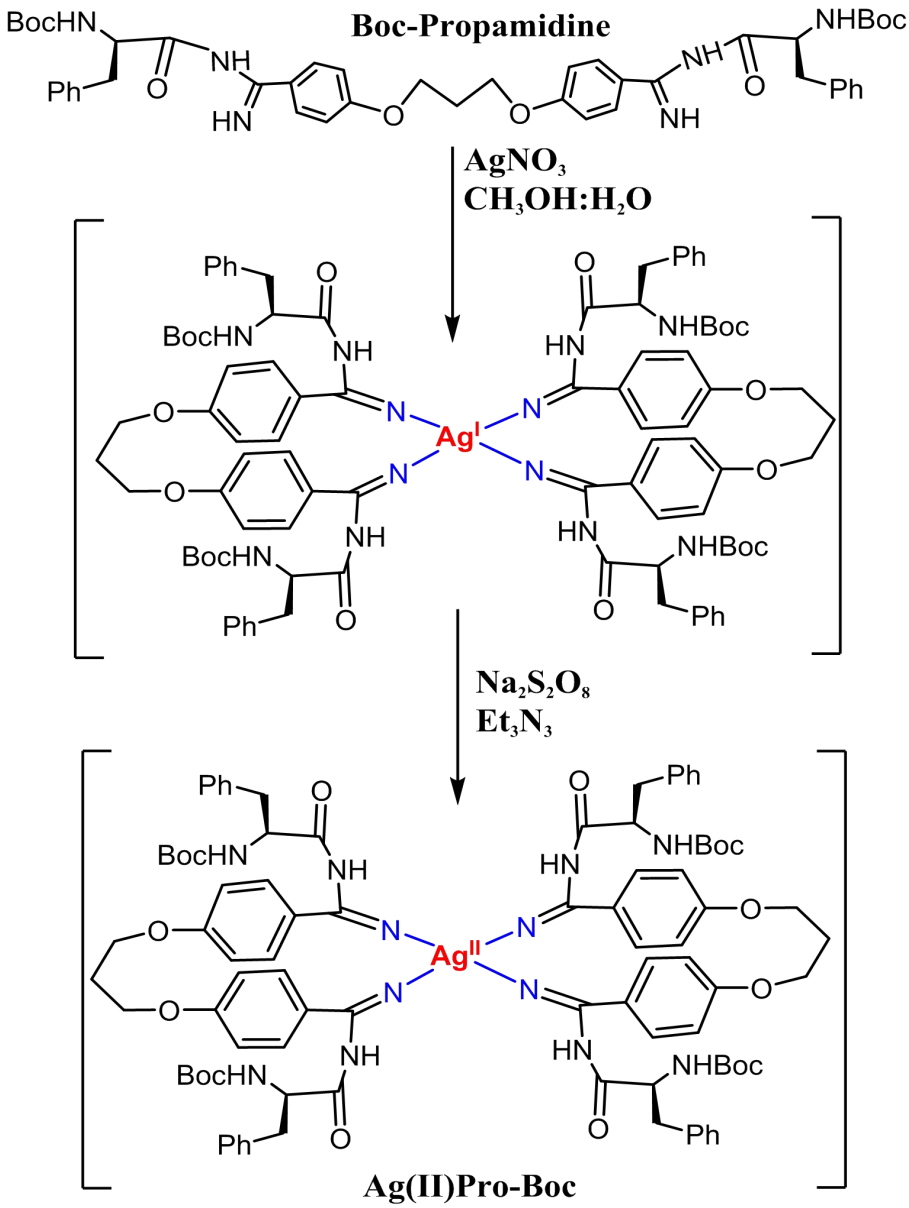


**Figure2.** Preparation Scheme for the Synthesis of Ag(II)Pro-Boc Complex

### 3. Synthesis of Ag(II)Pro-Boc Complex

The synthesis of Ag(II)Pro-Boc complex procedure is shown in figure 2. The complex was synthesized in water: methanol (4:1) solvent system. BOC-Propamidine, $AgNO_3$ and sodium persulfate were used at a 2:1:1 molar ratio to form the complex. 0.2mmol of BOC-Propamidine was first dissolved in solvent, followed by the pH of the reaction mixture was adjusted by adding triethylamine. The reaction medium was stirred for 20 minnutes after adding 0.1 mmol $AgNO_3$ solution. Before stirring the reaction mixture, sodium persulfate solution was added. Reaction was stirred at room temperature for another 30 mins. Then the compound was filtered by vacuum filtration and washed with methanol to remove the unreacted reagents and impurities. Dried the product and characterized it by $^1H$ NMR, Mass spectroscopy, FT-IR and UVs.

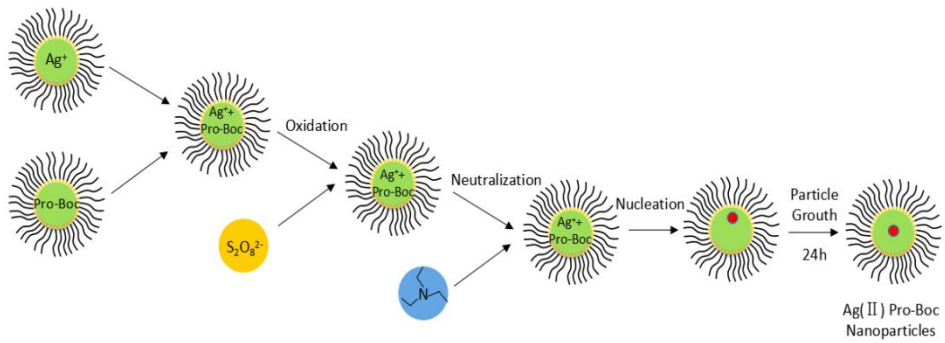


**Figure3.** Schematic Representation of the Synthesis of Ag(II)Pro-Boc Nanoparticles by Reverse Microemulsion Technique

## 4. Synthesis of Ag(II)Pro-Boc Complex Nanoparticles

Silver complex nanoparticles were synthesized using a water in oil microemulsion technique[21]. The synthesis of Ag(II)Pro-Boc complex nanoparticles procedure is shown in figure 3. Briefly, the nanoparticles were prepared by dissolving 0.44g of AOT (0.1M) in 10 ml of heptane, followed by addition of 90 µl Boc-Propamidine aqueous solution. Afterwards, 90 µl of silver nitrate and 90 µl of sodium persulfate were added to the heptane solution simultaneously. The pH of the reaction medium was adjusted by adding triethylamine to form a stable microemulsion (pH=7.0). In this mixture reaction, silver nitrate, Boc-Propamidine, and sodium peroxydisulfate were used at a 1:2:2 molar ratio to form the complex. The reaction mixture was stirred at room temperature for 1 h and microemulsion system turned brown which indicates higher valence silver state formed. Then the reaction was stirred for 24 h at room temperature. To isolate nanoparticles ethanol was used to break the microemulsion system. And nanoparticles were collceted by centrifugation at 10000 rpm for 10 minutes. After centrifugation, the nanoparticles were washed twice using methanol to remove the unreacted starting materials and impurities. Finally, silver complex nanoparticles were dried by vacuum, collected and reserved at room

temperature.

### 5. Synthesis of Silver Nanoparticles

20 nm of silver nanoparticles were synthesized according to the previously published method[22]. Finally, the color of the solution changed to yellow. Cool the nanoparticles solution to room temperature and centrifuge the suspensions at 12000 rpm for 15 minutes. Then, wash the nanoparticles twice using the distilled water to remove the unreacted starting materials and impurities. The products were characterized by TEM and UV-Vis spectroscopy.

### 6. Transmission Electron Microscope (TEM) Analysis

The size and morphology of silver complex nanoparticles were measured using transmission electron microscope (TEM). The sample for TEM characterization was prepared by adding 10ul the nanoparticle-containing solution on 200-mesh carbon-coated copper grids. Afterwards, keep it dry enough at room temperature for further analysis.

### 7. Characterization of Silver--Boc-Propamidine Complex

For Boc-Propamidine (ν, $cm^{-1}$): 3326 (-CONH-), 1680 (C=N, m) and 1607(C=C), 1497(-$CH_2$) 1251(C-N). For Ag(II)Pro-Boc Nano (ν, $cm^{-1}$): 3320 (-CONH-), 1605 (C=C),

2348 (C-N, m), 1507(-$CH_2$),1259(C-N ).

### 8. Bacterial Strains and Culture Condition

The tested organism strains include three strains of gram-positive bacteria (Staphylococcus aureus [ATCC 25923], Enterococcus faecalis [ATCC 29212], Streptococcus agalactiae [ATCC 12386]) and three strains of gram-negative bacteria (Pseudomonas aeruginosa [ATCC 27853], Klebsiella pneumonia [ATCC 10031], Acinetobacter calcoaceticus [ATCC 23005] ). Six strains of organism were obtained from American Type Culture Collection (Rockville MD, USA). All tests were performed in accordance with the Clinical and Laboratory Standards Institute guidelines. The six bacterial strains were cultured overnight for the purpose of inoculation. The strains were grown in Muller Hinton Broth or Muller Hinton Agar (MHB, MHA, Difco Laboratories, Detroit, MI, USA) at 37 °C under aerobic conditions.

### 9. Determination of MICs and MBCs

Minimum inhibitory concentrations (MICs) were determined by broth microdilution method in Muller Hinton Broth (MHB) for six bacterial strains using Multi-Reader (Molecular Devices, Spectra MAX M5). 96 well microplates were read after 24h

incubation at 37 °C under aerobic condition. Organism strains were cultured in 96 well microplate. In order to evaluate the inhibitory effect of antimicrobial agents on bacterial growth, each well was provided with different concentrations of antimicrobial agents. Microbial growth was examined following 24 h incubation in air at 37 °C, and MICs was defined as minimum concentration of antimicrobial agents to prevent bacterial growth. The effect of coagulation was also determined in the uninoculated group. The minimum bactericidal concentrations (MBCs) were determined by the reinoculation from wells onto empty Muller Hinton Agar plates. After 24 h incubation, the MBCs was defined as the lowest concentration that no bacteria growth was observed on the agar plates. All tests were performed in triplicate. The determination of MICs and MBCs is based on the guidelines recommended by the Clinical and Laboratory Standards Institute (CLSI).

# Ⅲ. Results and Discussion

## 1. Synthesis of Ag(II)Pro-Boc Nanoparticles

The synthesis of high valence silver-Boc-propamidine complex nanoparticles is shown in Figure 3. The main step is to determine the appropriate solvent medium. Ag(II)Pro-Boc complex was synthesized under different solvent conditions. Initially, we attempted different solvent conditions such as dimethyl sulfoxide (DMSO), water, and methanol in the process of synthesis. Equal and different proportions of solvents were also examined. Finally, we successfully performed the reaction in water:methanol (4:1) solvent system. We aquired high valence silver complex using sodium peroxysulfate to oxidize the silver ion. Triethylamine was added to a neutral homogeneous microemulsion medium for complexation reaction. Under neutral conditions (pH = 7) to maintain an appropriate medium for 24 h to achieve fine and uniform size of nanoparticles.

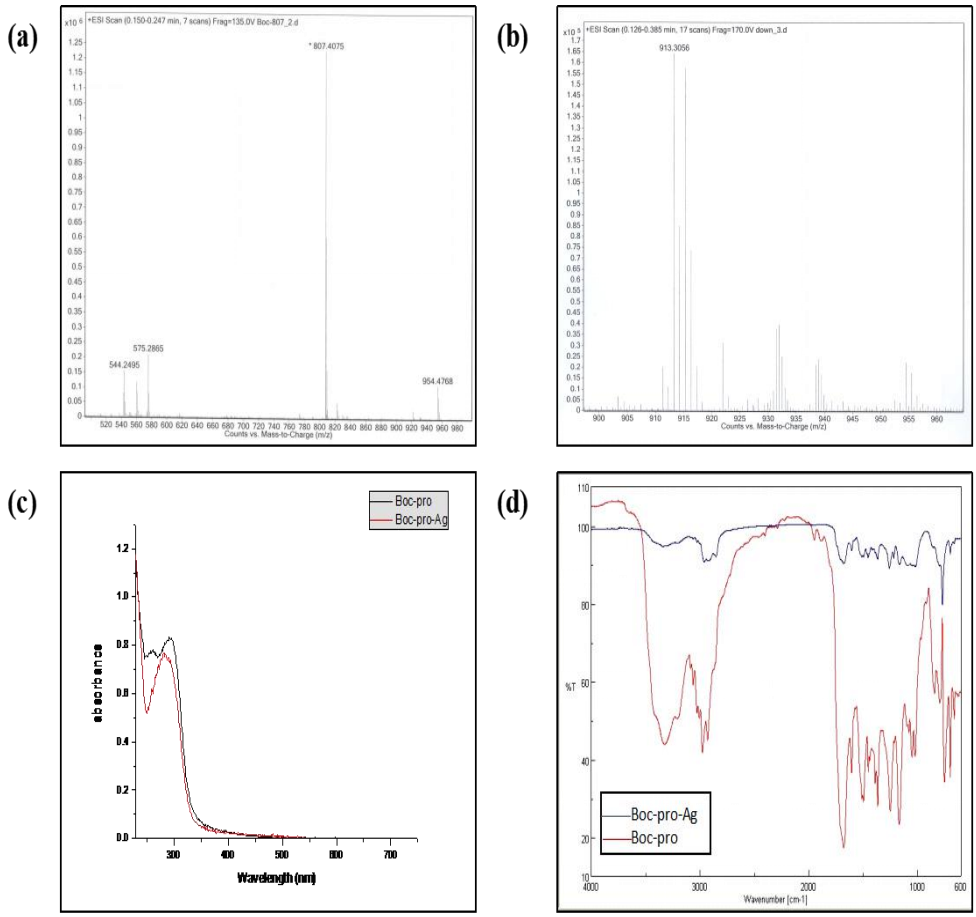


**Figure4.** **(a)** ESI-Mass Spectra for Boc-Propamidine; **(b)**ESI-Mass Spectra for Ag(II)Pro-Boc; **(c)** UV-Vis Spectra of Boc-Propamidine (black) and Synthesized Ag(II)Pro-Boc Nanoparticles (red); **(d)** IR Spectra of Boc-Propamidine (red) and synthesized Ag(II)Pro-Boc Nanoparticles ( black)

## 2. Charecterization of Ag(II)Pro-Boc Nanoparticles

Figure4.a and Figure4.b show the ESI-MS of synthesized Boc-propamidine and Ag(II)Pro-Boc nanoparticles. ESI-MS confirmed the formation of Boc-propamidine ($C_{45}H_{54}N_6O_8$, m/z: 807). Moreover, the silver and Boc-propamidine complex formation was vertified by ESI-MS spectrum. The fragmented mass of Ag(II)Pro-Boc was observed at 913 ($C_{45}H_{53}AgN_6O_8$ =913 g/mol). The UV absorption spectroscopy is commonly used to characterize the nanoparticles. The absorption pattern of Ag(II)Pro-Boc nanoparticle is different from the starting material Boc-Propamidine ligand. The Ag(II)Pro-Boc nanoparticles showed strong absorption peak at 270 nm with characteristic peaks at 320nm as shoulder peaks (Figure4.c). FT-IR Spectrum of Ag(II)Pro-Boc complex are shown in Figure4.d The band at 3320 and 3028 $cm^{-1}$ appeared in IR spectrum are the characteristic broad peaks of amide (-CONH-) and imine( =NH) of the starting material Boc-propamidine. In the IR spectra of the compound (Ag(II)Pro-Boc), this broad imine band disappeared which clearly indicated the formation of the complex (Figure4.d).

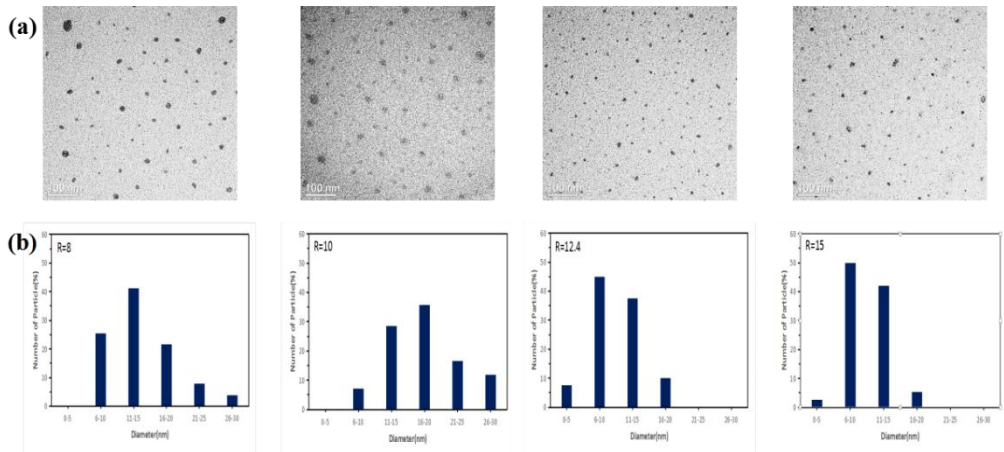


**Figure5.** **(a)** TEM Images of Ag(II)Pro-Boc Nanoparticles Synthesized at Different Water-to-Surfactant Molar Ratios (R = 8, 10, 12.4 and 15). Scale bar is 100 nm; **(b)** Size Distribution and the Average Diameters of Ag(II)Pro-Boc Nanoparticles at Various R Values (8, 10, 12.4 and 15 are 13.6, 16.8, 10.6 and 8.9 nm, respectively).

For the synthesis of Ag(II)Pro-Boc nanoparticle, we applied a microemulsion composition containing water / AOT / heptane ternary system. The molar ratio of various water to surfactant (R) is 8, 10, 12.4, 15 respectively. Figure 5.a and Figure5.b show the transmission electron microscopy (TEM) image and the size distribution of the Ag(II)Pro-Boc nanoparticles prepared at different R values. TEM images demonstrate that the particles exhibit a monodisperse spherical morphology whose initial size increases and then decreases corresponding to an increase in R value.

The diameter mean size is 13.6 nm for R = 8, and average particle diameter increased to 16.8 nm , at R = 10. There is a continuous reduction in the mean size to 10.6 nm for R = 12.4 and 8.9 nm for R = 15. On the whole, the size of the nanoparticles increases with the increasing of R value, which means the amount of water droplets in microemulsion. Thus, the increase in the mean sizes of Ag(II)Pro-Boc nanoparticles with the increase in R value from 8 to 10. However, the mean size of the nanoparticles reduced when R value is 12. We observed that as the diameter of the microemulsion increases the average particle size decreases further when R value is 15. From these results it can be seen that the microemulsion droplets provide a limited microenvironment while other parameters may

play a critical role in influencing the sizes of the Ag(II)Pro-Boc nanoparticles formed in the microemulsion[5].

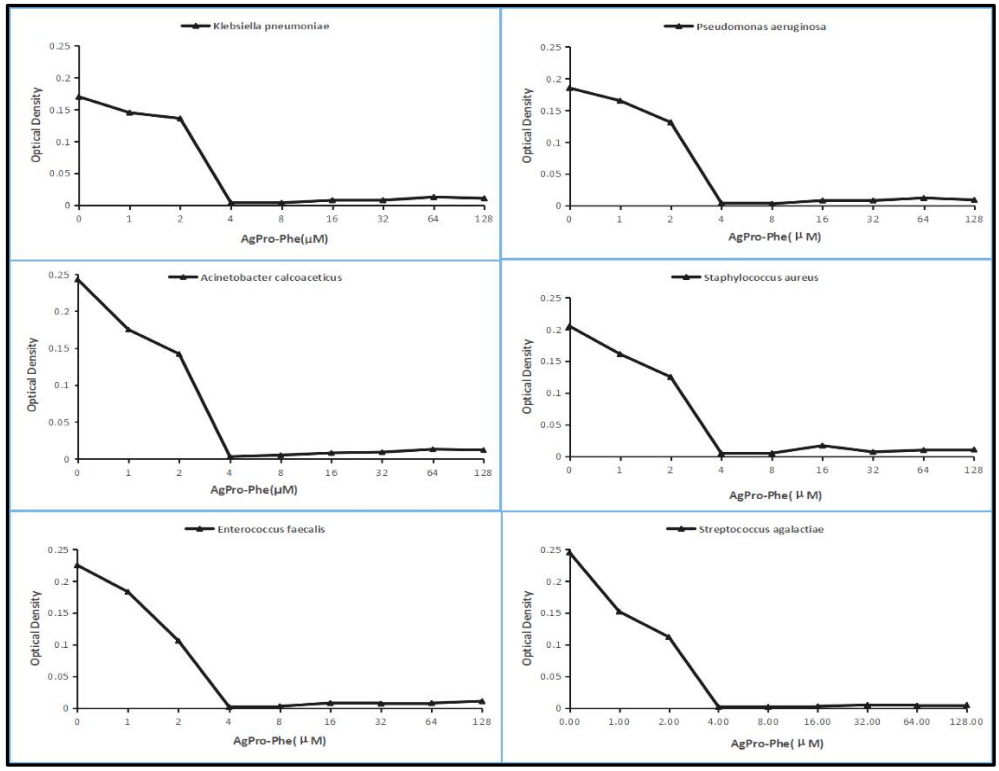


**Figure6.** The Minimal Inhibitory Concentrations (MICs) were Determined by Measuring the Optical Density Using Multi-Plate Reader.

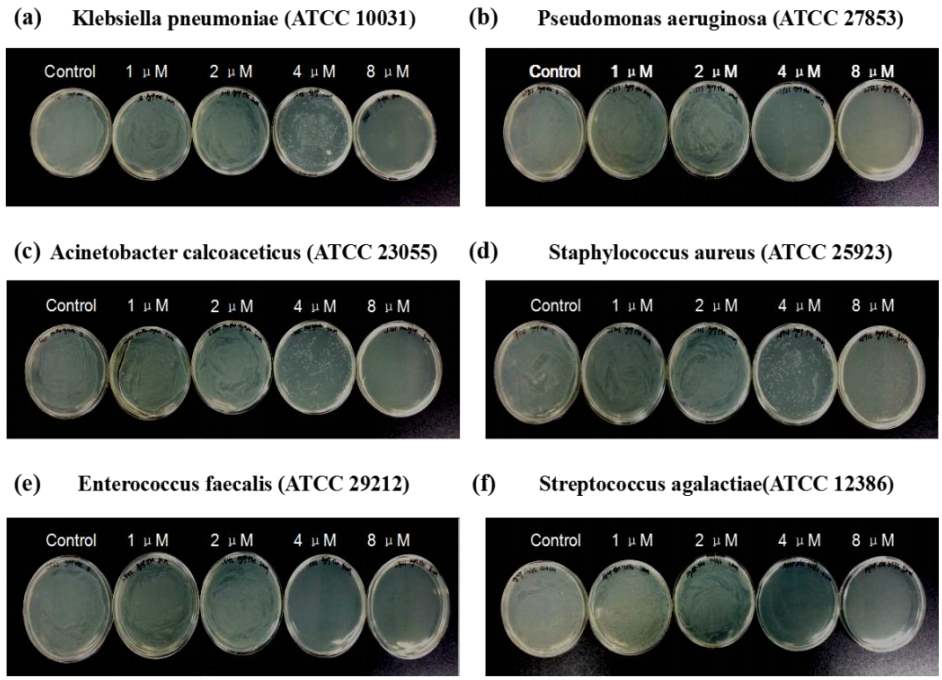


**Figure7.** After Measuring the Optical Density, Minimum Bactericidal Concentrations (MBCs) were Determined by the Reinoculation Gram negative/ Positive strains (a, b, c, d, e, f) from Wells onto Empty Muller Hinton Agar(MHA), Supplemented with Different Concentrations of Ag(II)Pro-Boc Nanoparticles, from Left to Right Control 1, 2, 4, 8 μM.

### 3. Antibacterial activities of Ag(II)Pro-Boc nanoparticles

We tested the antibacterial activities of the Ag(II)Pro-Boc nanoparticles toward gram-positive and gram-negative responsible microorganism strains for the DFUs. Determination of minimal inhibitory concentrations (MICs) is a standard microbiological technique for the evaluation of antimicrobial activity. MICs were defined as lowest concentration of antimicrobial agents to inhibit visible bacterial growth after 24h incubation. The MICs values presented in Table were determined using a broth microdilution method. The minimum bactericidal concentrations (MBCs) were determined by the reinoculation from wells to empty Muller Hinton agar (MHA) plates. The MBCs was defined as the lowest concentration that no bacteria growth was observed on the agar plates after 24 h incubation. The MBCs values were determined to evaluate the bactericidal properties of Ag(II)Pro-Boc nanoparticles.

The MICs results after measuring the optical density through the use of 96 well multi-microplate reader were shown in Figure6. From the data, the synthesized Ag(II)Pro-Boc nanoparticles has the best inhibitory effect on all strains. The MICs values of Ag(II)Pro-Boc nanoparticles were in the range of 4 μ M according to the optical density.

These results indicate that there is no difference in MIC values between gram positive and gram negative bacteria. For MBCs values, apart from the MBCs of enterococcus faecalis and streptococcus agalactiae are 4 μ M, the other strains MBCs all are 8 μ M(Figure7).

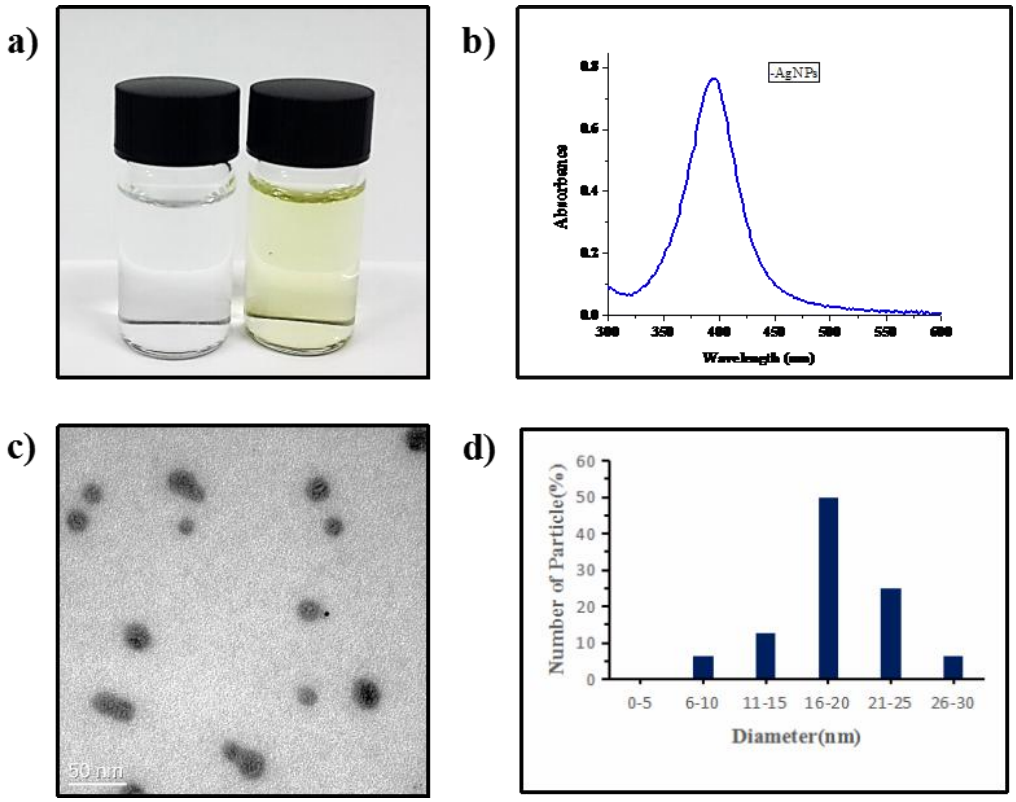


**Figure 8.** (a) Visual Observation of Synthesized AgNPs (left) before and (right) after Addition of Reducing Agent, the Color Changes to Yellow; (b) UV-Vis Spectra of AgNPs; (c) TEM Image , Scale bar is 50nm ;(d) Particle Size Distribution of AgNPs.

## 4. Comparison of Antibacterial Activities between Ag(II)Pro-Boc Nanoparticles and Silver Nanoparticles

We compared antibacterial activities between Ag(II)Pro-Boc and silver nanoparticles with similar particles size distribution. The UV-Vis absorption spectra and TEM of AgNPs as well as size distributions were shown in figure8. The TEM and size distribution study confirmed that they were in same size distribution. The values of MICs and MBCs the Ag(II)Pro-Boc nanoparticles and silver-only nanoparticles were determined. The results was shown in Table 1. As shown in the results, the antibacterial activities of Ag(II)Pro-Boc nanoparticles are approximately 5 to 10 times stronger than silver-only nanoparticles.

| Types of Bacteria | Ag(II)Pro-Boc NPs | | AgNPs | |
|---|---|---|---|---|
| | MIC (μM) | MBC (μM) | MIC (μM) | MBC (μM) |
| Klebsiella pneumonia | 4 | 8 | 20 | 40 |
| Pseudomonas aeruginosa | 4 | 8 | 40 | 40 |
| Acinetobacter calcoaceticus | 4 | 8 | 20 | 40 |
| Staphylococcus aureus | 4 | 8 | 40 | 80 |
| Enterococcus faecalis | 4 | 4 | 40 | 40 |
| Streptococcus agalactiae | 4 | 4 | 20 | 40 |

**Table1.** Antibacterial Activity of Synthesized Ag(II)Pro-Boc Nanoparticles and Silver-Only Nanoparticles against Pathogens of Diabetic Foot Ulcers.

# Ⅳ. Conclusions

In this study, we synthesized silver(II) Boc-propamidine nanoparticles by reverse microemulsion method and it has been characterized by spectroscopic techniques. We determined the antimicrobial activity of silver(II) Boc-propamidine nanoparticles against both gram-positive and gram-negative pathogenic bacteria of diabetic foot ulcers. Compared to silver nanoparticles, Ag(II)Pro-Boc nanoparticles show a more favorable antibacterial effect. Because of its stability, uniform dispersity and high antibacterial activity, these nanoparticles are expected to become the next generation of topical antimicrobial agents for the diabetic foot ulcers care.

# Ⅴ. References


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# Abstract

Diabetic foot ulcers(DFUs) is one of the main complications of diabetes mellitus, which seriously affects the health of diabetic patients. Wound healing is an essential process in the treatment of DFUs. The inflammatory with the infection of pathogenic bacteria is one of the important dynamic phases in wound healing. Antibiotics has found a widely utilization in DFUs. Due to antibiotic resistance, silver containing antimicrobial agents deserve greater attention and appear to be effective. To address this problem, we designed and synthesized the high valence silver complex nanoparticles using reverse microemulsion technique for its higher antibacterial ability. Proliferation is another essential step in the wound healing. Wound healing increases the requirement for amino acids for repair and cell growth. L-Phenylalanine is one of the essential amino acids, which means it cannot be synthesized in the human body and must be supplemented by diet. In this study, we conjugate propamidine with Boc-L-phenylalanine which contain L-phenylalanine moiety as a ligand for the silver complex synthesis. Thus, this compound possesses the proliferative potential therapeutic ability due to containing amino acid moiety. Silver(II)-Boc-propamidine nanoparticles have been characterized by spectroscopic

techniques. Up to now, we investigated the antibacterial activity of silver(II)-Boc-propamidine nanoparticle against pathogens of DFUs. The newly synthesized high valence silver complex nanoparticles demonstrate better antibacterial activity compared to silver nanoparticles (AgNPs). This study concludes the synthesized silver(II)-Boc-propamidine nanoparticles show better antibacterial ability. And it may serve as next generation therapeutic agent for the treatment of diabetic foot ulcers.

**Key words**: Propamidine, Silver Nanoparticle, Diabetic foot ulcers, Antibacterial, Proliferation.