MUSSEL-INSPIRED SYNTHESIS OF ANTIBACTERIAL PAPER BASED ON SILVER NANOPARTICLES

A thesis submitted in partial fulfillment of the requirement for the degree of M.Sc. in chemistry

SUBMITTED BY

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Declaration by the Candidate

I do hereby declare that this thesis work, entitled "Mussel-Inspired Synthesis of Antibacterial Paper Based on Silver Nanoparticles" submitted in partial fulfillment for the requirement of M.Sc. Degree in Chemistry to the Department of Chemistry, Bangladesh University of Engineering and Technology (BUET) in November 2016, is genuine work done by me under the supervision of Dr. Md. Shafiul Azam, Assistant Professor, Department of Chemistry, Bangladesh University of Engineering and Technology (BUET). The thesis or part of it has not been submitted before in any degree or diploma.

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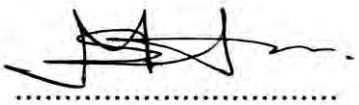
Certificate

This is to certify that this thesis work entitled "Mussel-Inspired Synthesis of Antibacterial Paper Based on Silver Nanoparticles" submitted by Md. Shafiqul Islam, Student ID: 1014032705, to the Department of Chemistry, Bangladesh University of Engineering and Technology (BUET) in partial fulfillment for the requirement of M.Sc. in Chemistry in November 2016 is based on his original research and investigation carried out under my guidance and supervision.

Supervisor:



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Certification of Thesis

A thesis on

MUSSEL-INSPIRED SYNTHESIS OF ANTIBACTERIAL PAPER BASED ON SILVER NANOPARTICLES

BY

Md. Shafiqul Islam

has been accepted as satisfactory in partial fulfillment of the requirements for the degree of Master of Science (M.Sc.) in Chemistry and certify that the student has demonstrated a satisfactory knowledge of the field covered by this thesis in an oral examination held on November 14, 2016

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November 2016 Md. Shafiqul Islam

Dedicated to
My Beloved parents
&
Honorable supervisor

Abstract

In this research, we developed a bio-inspired strategy for immobilizing silver nanoparticles (AgNPs) onto cellulose paper to constract antibacterial paper. We exploited interesting dopamine chemistry to achieve *in situ* reduction of Ag^+ salt to AgNPs and deposition of AgNPs on the paper surface. Firstly, the cellulose paper surface was chemically modified with carboxylic acid groups by reaching it with succinic anhydride. Next, the carboxylic groups of the surface were reacted with the $-NH_2$ group of dopamine in a buffer to introduce the dopamine molecules. The covalently attached dopamine molecules on the paper surface resulted in rapid and efficient immobilization of AgNPs from the solution of $AgNO_3$. The loading and the robustness of AgNPs into paper matrix were quite excellent. The paper showed excellent antimicrobial activity against Vibrio pharahaemolyticus strain 2A1, Vibrio pharahaemolyticus strain 2A2, Enterococcus faecalis strain FF11, Enterococcus faecalis strain F1B1, Serratia marcescens strain 4V3 which are highly virulent and resistant to multiple antibiotics. The paper also showed antifungal activity against extremely virulent "wheat blast" disease causing fungal species. Phytotoxicity test reveals that the synthesized AgNP-paper does not cause any harm to seed germination and plant leaves cells. The paper was characterized by x-ray photoelectron spectroscopy (XPS), Scanning electron microscopy (SEM), Atomic-force microscopy (AFM) and Fourier transform infrared spectroscopy (FTIR) analyses. From the SEM and thermal study, it was found that the deposition of AgNPs into paper matrix was time dependent. The AgNPs which is deposited onto paper matrix had an average size of 50-60 nm and were uniformly distributed on the every part of the paper surface. The XPS analysis revealed that the binding energy peaks at 366.0 and 372.0 KeV correspond to Ag (0). No additional Ag compounds were detected during the XPS analysis.

Contents

List of Figures

List of Tables

CHAPTER 1
INTRODUCTION

1.1 General Remarks

Before the discovery of bacteria, virus and other microorganisms there was some animus belief spreading all around the world about infectious diseases. People believed that diseases such as cholera, chlamydia are happened because of "bad air" till 19th century [1]. In ancient time, people of China used to treat wounds by using frog skin and they believed that the skin has some kind of magical power which is responsible for the healing. But later scientist found that the frog skin contain some antimicrobial peptides which is responsible for prevention of further bacterial infection. The use of turmeric, honey, alcohol is also well known medicine as wound healing. In this way, people of different civilizations began to create their own herbal medicinal treatments for wounds depending on the trees, shrubs, or any other type of plants located in their environment. After the discovery of bacteria in 1670s by van Leeuwenhoek and further some elegant experiments by the French scientist Pasteur people all around the world try to believe that diseases happen due to the invading of some virulent microorganisms. From then scientists all over the world try to explore the way of the spreading and prevention of microorganisms like bacteria, virus, fungus etc. from contaminated environment. One of the best ways of prevention and spreading of the microorganism is the use of antibacterial materials which is investigating time to time as the knowledge of microorganism increases. In this way, the new types of antibacterial or antimicrobial materials are developed to fight against new type of microorganism species. One of the antibacterial materials is silver which was used as prevention of bacterial growth from ancient time. Ancient people preserve their rotting food or drink by putting them in silver container. But they don"t know how or why the silver pot retained the food quality. But recent reports have shown that it is the silver nanoparticles which show antibacterial activity against a broad spectrum of bacterial species. Thus, the holding of food quality in silver pot was responsible for releasing of silver nanoparticles into the food media. Therefore, immobilization of silver nanoparticles has widely investigated all over the world for making various products such as hand wipe, wrapping material and medical materials etc. Herein, we report a novel strategy to synthesize a planar and flexible antibacterial paper by depositing AgNPs onto it.

1.2 Antibacterial materials

An antibacterial material is a product that can kill or inhibit bacteria such as *E. coli, Staphylococci, Salmonella* etc. that cause skin infections, food poisoning, intestinal illnesses

and other commonly transmitted diseases, On the other hand, an antimicrobial material is a product that kills (and prevents the growth or reproduction of) not just bacteria, but other potentially harmful microscopic organisms such as protozoa, yeast, fungi, viruses, some algae and some worms as well. An antimicrobial agent is an antibiotic, an antibacterial, an antifungal, an antiparasitic and an antiviral agent all-in-one. There are hundreds of antibacterial materials found in the market for different purposes. They are using in home, industry, clinic etc. for different purposes in our daily life. Basically, the antibacterial materials used in different products may be based on nanomaterials, synthetic chemical compounds, planer surface etc.

1.2.1 Synthetic chemical compounds

The necessity of hand washing with antibacterial agents was probably started with Ignaz Philipp Semmelweis, a Hungarian obstetrician who discovered that how hand washing and cleanliness in medical procedures prevents maternal deaths [2]. In the mid 20th century the development of polymer synthetics was established for wound dressings and then the use of nylon, polyethylene, polypropylene, and polyvinyls was started for medical purposes. Today we are using thousands of organic compound based antibacterial materials for various purposes at home, factory, hospital etc. For cleaning purposes we generally use soap, detergent, sanitizers, wipes which contain antibacterial agent such as alcohols, aldehydes, halogen-releasing compounds, halophenols, bisphenols and quaternary ammonium compounds etc. For antiseptic and disinfection purposes peroxides, acids, gaseous substances, anilides, biguanides, heavy metals compounds, phenols and cresols, dyes or tints, detergents, derivatives of different chemical groups, agents from plant source are generally used in different products. Some of the disinfectant and antiseptic chemicals are hydrogen peroxide, potassium permanganate, ozone, peracetic acid, salicylic acid, boric acid, ethylene oxide, formaldehyde, glutaraldehyde, triclocarban, chlorhexidine, alexidine, polymeric biguanides, Phenol, cresol, resorcinol, vagotil, cetrimide, benzalkonium chloride, cetylpyridinium chloride, chlorinated lime, chloramine B, chlorhexidine, iodinole, iodovidone, silver nitrate, zinc sulfate, copper sulfate, brilliant green, rivanol, methylene blue, roccal, aethonium, cerigelum, decamethoxinum, furacilinum, novoimaninum, chlorophylliptum, and lysocim etc. [3,4]. But all the chemicals are not equally safe, effective and popular. Disinfectants are usually used to inhibit or kill microorganisms on inanimate objects like walls, floor, air, and medical tools. The process of disinfection prevents infection by reducing the number of

potentially infective organisms either by killing, removing, or diluting them. Antiseptics are sufficiently low toxicity for host cells that they can be used directly on skin, mucous membranes, or wounds. Disinfectants and antiseptics can be used both for disinfecting of medical tools and human skin depending on their concentration. Besides, the disinfectant and antiseptic agents there are huge amounts of antibacterial materials used in agricultural purposes. Different types of organic, aromatic, inorganic and heterocyclic compounds are employed as antibacterial agents. The chemicals may be used for this purposes are salts of toxic metals and organic acids, organic compounds of chlorine and sulfur, quinones and heterocyclic oxygen compounds. All these antibacterial or antimicrobial agents are used for household, industrial and medical purposes. But all these are not useful or effective for all kinds microorganism. Although we have used these antibacterial or antimicrobial agents in our daily life, there are some problems with these agents. These materials are usually known to be associated with bacterial and compounded antibiotic resistant, skin irritation, allergy susceptibility, dioxin contamination to destruction of fragile aquatic ecosystems, environmental pollution, relatively complex processing, and high cost [5, 6, 7]. Therefore, the investigation of new antibacterial materials is still carry on.

1.2.2 Nanomaterials

Recently, nano-sized materials have grown much attention to the scientific community because of their different type of unique property which is not found in the bulk matter. For example, materials such as silver, gold, which are chemically inert at normal scales, can serve as a potent antibacterial and chemical catalyst at nanoscales. The particles or materials which have at least one of the dimensions in 1-100 nm is called nanomaterials. Materials that have one dimension in the nanoscale are layers, such as thin films or surface coatings. Materials that are nanoscale in two dimensions include nanowires and nanotubes. Materials that are nanoscale in three dimensions are particles, for example precipitates, colloids and quantum dots. Nanocrystalline materials, made up of nanometre-sized grains, also fall into this category. There are lots of nanomaterials which show antibacterial activity that have reported for the last few decades are Titanium dioxide [8], Manganese oxides [9], zinc oxide nanoparticles[10], silver nanoparticles [11], gold nanoparticles [12], copper nanoparticles [13], iron oxide nanoparticles [14], carbon nanotubes [15] etc. In some case, these nanomaterials show much more efficiency against microorganisms than the antibiotics. Some of them are efficient against antibiotic resistant strains. Therefore, these materials are now

widely investigated for exploiting different purposes. We know that the earlier a disease can be detected, the easier it is to remedy. To achieve this, research is focusing on introducing into the body specially designed nanoparticles. These nanoparticles are composed of tiny fluorescent 'quantum dots' that are 'bound' to targeting antibodies. In turn, these antibodies bind to diseased cells. When this happens, the quantum dots fluoresce brightly. This fluorescence can be picked up by new, specially developed, advanced imaging systems, enabling the accurate pinpointing of a disease even at a very early stage. Researchers are attempting to use nanotechnology-based techniques to develop new methods for fighting antibiotic resistance bacterial infections. Nanoparticles can help fight staph infections, burns, and other conditions eradicating or avoiding bacterial infection. It's possible that these nano-techniques could remove bacterial infection in minutes, rather than in weeks as is currently the case with antibiotics. Researchers are developing a technique to kill bacteria using gold nanoparticles and infrared light. This method may lead to improved cleaning of instruments in hospital settings. Another attempt is investigating the use of quantum dots to treat antibiotic resistant infections. Researchers are investigating the use of polymer coated iron oxide nanoparticles to treat chronic bacterial infections. One of the earliest nanomedicine applications was the use of nanocrystalline silver which is as an antimicrobial agent for the treatment of wounds.

1.2.3 Planar surfaces

For exploiting in various settings including clinics, industry, and even the home antibacterial surfaces are being popular recently. The most common and most important use of antimicrobial coatings has been in the healthcare setting for sterilization of medical devices to prevent hospital associated infections, which have accounted for almost 100,000 deaths in the United States [16]. An antibacterial surface is a surface which contains an antibacterial agent that kills or reduces the growth of microorganisms [17]. Different processes are being employed to functionalize the antibacterial surfaces depending on the nature of the surfaces. One way is to coat directly the surface with antibacterial materials that is toxic to microorganisms and another way is to embed the antibacterial materials by tailoring or applying some chemical method onto the desired surface. The metallic copper and its alloys such as brasses, bronzes, cupronickel, copper-nickel-zinc, and others planar surface show antimicrobial property and destroy a wide range of microorganisms [18, 19]. Apart from health industry, antimicrobial surfaces are being popular for getting self-cleaning surfaces

and can be used as air cleaning, water purification, and antitumor activity [20]. There are two ways of surface modification physical and chemical. In the physical method the surface is made in such a way that the microorganism is adhere to its surface easily such as textile surface. The topology of the textile surface has lots of interstitial spacing between fibers that is why it can easily adhere microbes onto its surface and no antibacterial agents is used in this case. On the other hand, in the chemical method chemical agents are used to modify the desired surface and therefore lots of different surface such as glass, graphene, paper etc. can be modified using this method. Generally, there are two approach of surface modification in chemical method such as "grafting to" and "grafting from". In the "grafting to" method the desired polymer or other substrate (may be antibacterial agent) is attached to the surface by coupling with the surface functional group and the end-functional of that polymer or substrate. In this case, a coupling agent is used to handle the reaction. Basically, grafting to method involves strong adsorption and chemical bond formation. A glass surface can be modified by pre-synthesized polymer such as PDMEAMA/PTMSPMA block copolymer simply immersing the surface in an aqueous solution containing the polymer [21]. For a process like this, grafting density depends on the concentration and molecular weight of the polymer as well as the amount time the surface was immersed in solution. In the "grafting from" approach the surface group is first modified with other functional group or molecules and then coupled with the desired end-functional group that would be useful for the preferred purposes. Here an initiator molecule is used which control the grafting reaction. Using these methods the desired surface can be given various properties such as superhydrophobic, antibacterial etc. However, coating of different antibacterial materials such as nanomaterials, anti-additives, Touch surfaces (copper and its alloy flim) also can change the normal surface into antibacterial. Generally, soft surfaces like paper surface are changed in that way. Other ways of changing a surface are wax solidification, lithography, vapor deposition, template methods, polymer reconfirmation, sublimation, plasma, electrospinning, sol-gel processing, electrochemical methods, hydrothermal synthesis, layer-by-layer deposition.

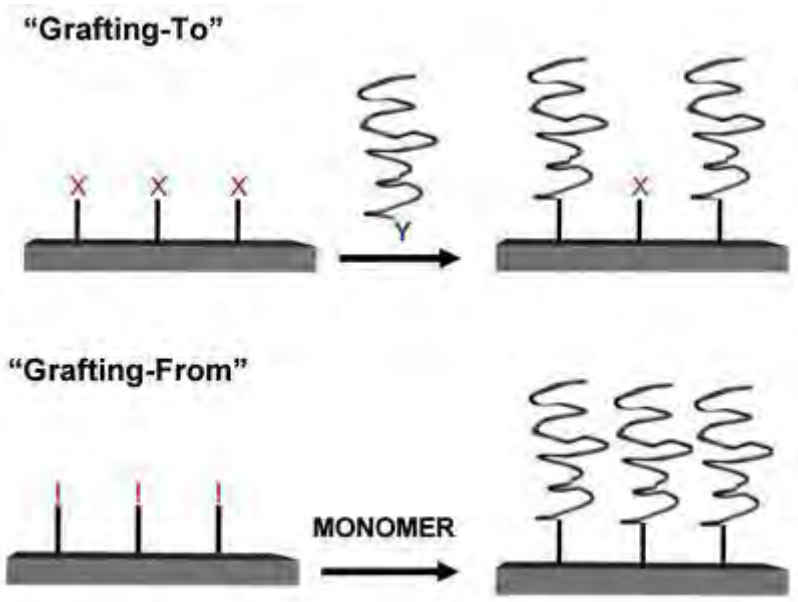

Fig 1.1 Schematic representation of „grafting to" and „grafting from" processes.

(Paula M Mendes, Chem. Soc. Rev., 2008, 37, 2512–2529)

1.3 Antibacterial paper

An alarming report of World Health Organization (WHO) 2013 said that, the developed countries like U.S there are at least 80,000 fatalities each year (About 200 deaths/day) from Healthcare Associated Infections (HAI). Patients may infected before admission to a hospital known as Community acquired infection or get infected inside the hospital known as Nosocomial infection/Healthcare infections not present nor Associated Infection. It includes infections that appear more than 48 incubating at time of admission, infections those acquired in the hospital but hours after admission and also occupational infections among staff appear after discharge. According to WHO 2013 report, the impact of HAI is far greater in the developing countries, with the risk being 2 to 20-fold higher than those in the developed world and prevalence studies report hospital-wide infection rates usually higher than 15%. In these countries, over 4000 children die of HAI every day. Approximately half of all patients admitted to neonatal intensive care units acquire an infection, and over half of them die. Scientific evidences showed that microbes causing HAI are most frequently spread between patients on the hands of health-care workers. A simple practice such as healthcare workers" hand washing/hand hygiene practices can improve the situation up to 51–83% was reported

by S. A. Creedon [22]. Hand hygiene is a simple, low-cost action to prevent the spread of the microbes that cause health care-associated infections (HAI). Some other facts we should know about bacteria are it can survive on average 20 minutes to 2 hours on hard surfaces such as desks, doorknobs and tables and a surface as small as a pinhead may contain up to 10 million bacteria and bacteria can double their number in 20 minutes. We can eliminate 90% of germs through proper hand washing. Wet hands spread 60,400 bacteria, while dry hand can only spread 200. Residual moisture left on improperly dried hands is the key factor for bacterial contamination and transmission. Thus drying of hands is a key factor in reducing the risk of infection. Acknowledging the above facts, researchers and company like cascade [23] have been developing a new paper which is known as antibacterial paper in order to exploit it in various setting especially in medical, food preservation. An antibacterial paper is used to prevent or reduce further bacterial contamination and transmission. A study was conducted by the University of Westminster, UK in 2009, to compare the levels of hygiene offered by Paper towels, Warm-air hand dryers and High-efficiency particulate arrestance (HEPA) filter jet-air hand. After washing, the hands which was dried with the warm-air dryer the total number of bacteria was found to increase on average on the finger pads by 194% and on the palms by 254%. Hands dried with the jet-air dryer, the total number of bacteria was found to increase on average on the finger pads by 42% and on the palms by 15%.When dried hands with a paper towel, the total number of bacteria was reduced on average on the finger pads by up to 76% and on the palms by up to 77%. Besides, hand hygiene the antibacterial paper can be used healthcare, laboratory, hospitality, education and government sectors. But unfortunately, the efficient number of antibacterial paper that is available, low cost, eco-friendly and non-toxic to human health is not found in the market. In the market, the commercially available antibacterial papers or towels are basically based on alcohols, surfactants and chlorinated aromatic compounds and inorganic compounds [24]. These papers cannot be used for all purposes such as food preservation. Therefore, researchers have still investigated to develop an efficient antibacterial paper. Recently, using nanotechnology scientists have achieved many amazing antibacterial paper such as fluorescent and magnetic antibacterial paper which can be used for bank notes /currency to prevent spread of infection through paper. For the medical purposes very few attempt have been made. One of the approaches is the graphene based [25] and another is silver nanoparticles based [26] antibacterial paper which have recently reported. But these are not commercially available.

1.4 Silver nanoparticles as antibacterial materials

Silver is used as a precious commodity in currencies, ornaments, jewelry, electrical contacts and photography, among others. This is because silver show as potent antibacterial agent that is toxic to bacteria, fungi, viruses and algae. Silver has long been used as a disinfectant; for example, the metal has been used in treating wounds and burns because of its broad-spectrum toxicity to bacteria as well as because of its reputation of limited toxicity to humans. In nanotechnology, a solid material becomes very small can‟t even seen in necked eye which increases its specific surface area and therefore increase its reactivity. The main thing of nanoparticles is that the electrons are now squeezed into small area which is known as quantum effect. This gives different physical and chemical property of nanosilver from the bulk. Due to the different physiochemical property of silver at nanometer scale is nowadays used in an increasing number of consumer and medical products. Silver nanomaterials are fine particles of metallic silver that have at least one dimension less than 100 nm. Nanosilver is not a new discovery. It has been known for over 100 years [27]. Previously, nanosilver or suspensions of nanosilver were referred to as colloidal silver. Before the invention of penicillin in 1928, colloidal silver had been used to treat many infections and illnesses [28]. If the bulk silver is converted into nanosilver, its effectiveness for controlling bacteria and viruses was increased multifold. This is because of the nanomaterials‟ extremely large surface area when compared to bulk silver resulting in increased contact with the bacterial and fungal species. When Nanosilver is in contact with bacteria and fungus, adversely affects the cellular metabolism of the electron transfer systems, and the transport of substrate in the microbial cell membrane. Nanosilver also inhibits multiplication and growth of those bacteria and fungi which caused infection, odor, itchiness and sores [29]. Nanosilver is an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria [30, 31, 32] including antibiotic-resistant strains [33, 34]. Gram-negative bacteria include genera such as *Acinetobacter, Escherichia, Pseudomonas, Salmonella,* and *Vibrio*. *Acinetobacter* species are associated with nosocomial infections, i.e., infections that are the result of treatment in a hospital or a healthcare service unit but secondary to the patient‟s original condition and Gram-positive bacteria include many well-known genera such as *Bacillus, Clostridium, Enterococcus, Listeria, Staphylococcus,* and *Streptococcus*. Antibiotic-resistant bacteria include strains such as methicillin-resistant and vancomycin-resistant *Staphylococcus aureus*, and *Enterococcus faecium*. Silver nanoparticles (diameter 5-32 nm, average diameter 22.5 nm) enhance the antibacterial activity of various antibiotics [35]. The antibacterial activities

of penicillin G, amoxicillin, erythromycin, clindamycin, and vancomycin against Staphylococcus aureus and Escherichia coli increase in the presence of silver nanoparticles [31]. Size-dependent (diameter 1-450 nm) antimicrobial activity of silver nanoparticles has been reported with Gram-negative bacteria and Gram-positive bacteria [36, 37, 38]. Small nanoparticles with a large surface area to volume ratio provide a more efficient means for antibacterial activity even at very low concentration. In addition to size and concentration, shape-dependent antimicrobial activity of silver nanoparticles has been shown with Gram-negative bacteria [39]. Silver nanoparticles of different shapes such as spherical, rod-shaped, truncated triangular nanoplates have been developed recently and were found that the truncated triangular silver nanoplates display the strongest antibacterial activity. This is because the truncated triangular silver nanoplates have large surface area to volume ratios and different types of crystallographic surface structures. The top basal plane of truncated triangular silver nanoplates is a high-atom-density surface i.e. a {111} facet. Generally, spherical silver nanoparticles may be cubo-octohedral, multiple-twinned decahedral, or quasi-spherical morphology which have {100} facets along with a small percentage of {111} facets whereas rod-shaped silver nanoparticles such as pentagonal rods have side surfaces with {100} facets and end with {111} facets [31, 40]. Silver reactivity is favored by {111} facets [41]. Spherical silver nanoparticles with {111} facets attach directly to the bacterial surface of the cell membrane and are located inside bacteria [37].

Nanosilver inhibits the formation of biofilms [42]. Biofilms are complex communities of surface-attached aggregates of microorganisms embedded in a self-secreted extracellular polysaccharide matrix. Biofilm forming bacteria act as efficient barriers against antimicrobial agents and the host immune system, resulting in a persistent colonization and infection at the site of the biofilm formation.

1.4.1 Antibacterial mode of action

Bacteria have different membrane structures which are the bases of their general classification as Gram-positive or Gram-negative. The key component of the cell wall peptidoglycan which is located immediately outside the cytoplasmic membrane imposes Structural differences. The cell wall of Gram-positive bacteria contains a peptidoglycan layer that is ~30 nm thick. Unlike the Gram-positive cell wall, the Gram-negative cell wall has only a thin peptidoglycan layer that is ~2-3 nm thick. In addition to the peptidoglycan layer,

the Gram-negative cell wall also contains an additional outer membrane composed of phospholipids and lipopolysaccharides, which face into the external environment.

Although the antimicrobial effect of silver ions has been studied extensively, the effects of nanosilver on bacteria and the bactericidal mechanism are only partially understood. Studies show that silver nanoparticles anchor to and penetrate the cell wall of Gram-negative bacteria. Therefore, it is reasonable to suggest that the resultant structural change in the cell membrane could cause an increase in cell permeability, leading to an uncontrolled transport through the cytoplasmic membrane and ultimately cell death [37, 43]. It has also been proposed that the antibacterial mechanism of silver nanoparticles is related to the formation of free radicals and subsequent free radical-induced membrane damage [44, 45]. A study of stress-specific bioluminescent bacteria was performed based on a synergistic toxic effect of the silver nanoparticles and found that they produce the silver ions [46]. The ions move into the cells and lead to the production of reactive oxygen species. Because of the membrane damage caused by the nanoparticles, the cells cannot effectively extrude the silver ions and limit their effect. Based on the greater tendency of silver ions to strongly interact with thiol groups of vital enzymes and phosphorus-containing bases [41] and on the presence of silver nanoparticles inside the cells [37], it is likely that further damage could be caused by interactions with compounds such as DNA. This interaction may prevent cell division and DNA replication from occurring, and also ultimately lead to cell death.

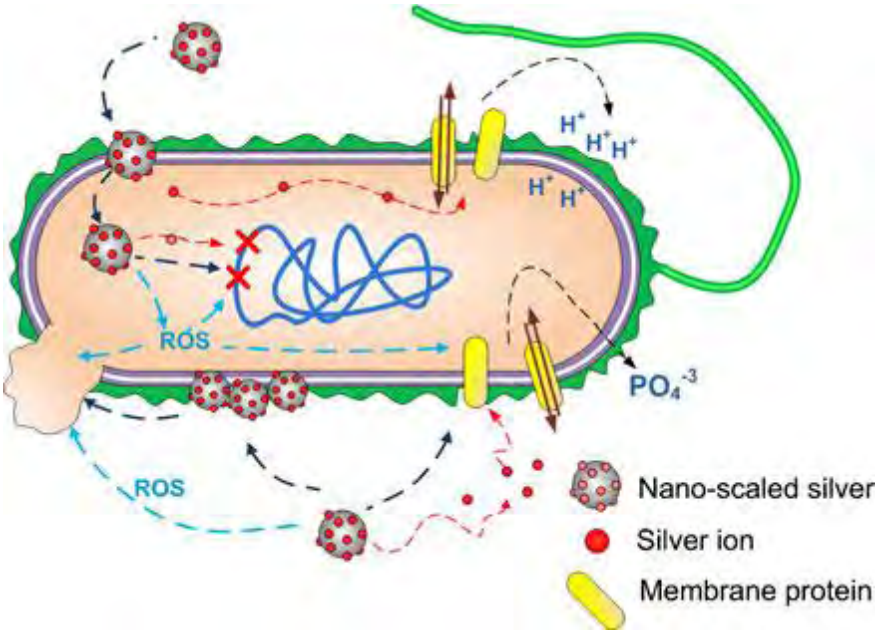

Fig 1.2 Diagram summarizing nano-scaled silver interaction with bacterial cells.

(Catalina Marambio-Jones, Eric M. V. Hoek *J. Nanopart Res.* **2010**, 12, 1531–1551)

Nanosilver is also an effective, fast-acting fungicide against a broad spectrum of common fungi including genera such as Aspergillus, Candida, and Saccharomyces. The exact mechanisms of action of silver nanoparticles against fungi are still not clear, but mechanisms similar to that of the antibacterial actions have been proposed for fungi [47]. Silver nanoparticles (diameter 13.5 ± 2.6 nm) are effective against yeast isolated from bovine mastitis [48]. Silver nanoparticles of diameter 5-20 nm and average diameter ~10 nm inhibit HIV-1 virus replication [49]. Gold nanoparticles (average diameter ~10 nm) showed relatively low anti HIV-1 activity (6-20%) when compared to silver nanoparticles (98%). Size-dependent antiviral activity of silver nanoparticles has been shown with HIV-1 virus [50]. Interaction of silver nanoparticles with HIV-1 was exclusively within the range of 1-10 nm.

That is why nanosilver is being incorporated in plastics, fabrics, paper, paint, and surface coatings. More than 200 products containing nanosilver are now available for public use [31].

1.4.2 Cytotoxicity effect

As silver nanoparticles are a potent material for various setting, the use of the material has increased day by day which increase the exposure of the material to the environment, human and aquatic life. Therefore, the effect of the silver nanoparticles has been studied widely. The toxicological data of metallic silver Ag (0) and monovalent silver Ag^+ is available in literatures. Silver has been used widely for its medicinal, antibacterial and antiviral properties for hundreds of years. There is relatively limited information available on its toxicity in the literature. Existing environmental and human studies suggest that some forms of silver, especially those that dissociate and release free silver ions are more toxic than others. Metallic silver has minimal risk to health. Various studies have shown that 1-5 g Ag^+/L is enough to kill sensitive aquatic and marine species [51,52]. The most common health effects associated with chronic exposure to silver are a permanent grey or blue grey discoloration of the skin which is known as argyria. Although, the concentration of silver used is not high enough to create risk to human health and environment. As Nanosilver has different size, surface area, solubility, ability to aggregate, chemical composition and surface chemistry from bulk silver, it should have different physicochemical properties and biological activities compared with the regular metal. It cannot be excluded that the increased reactivity of nanosilver (because of the large surface area) leads to increased toxicity due to the activity of free silver ions released by the nanoparticles. The health effects of the nanoparticles used in consumer products are not yet known, though various studies have revealed adverse health effects of materials previously considered safe. There are contradictory studies on silver nanoparticles and ion cytotoxicity from laboratories around the world. Some studies have shown that it is toxic to human and animal cells. As silver is known to have a lethal effect on bacteria, but the same property that makes it antibacterial may render it toxic to human cells. Concentrations of silver that are lethal for bacteria are also lethal for both keratinocytes and fibroblasts [53]. In vitro studies have demonstrated that nanosilver has effects on reproduction, development, and has an effect on DNA among others. Recent research with zebra fish showed that highly purified, single silver 12 nm nanoparticles affected early development of fish embryos [54]. Silver nanoparticles have the potential to cause chromosomal aberrations and DNA damage and are capable of inducing proliferation arrest in zebrafish cell lines [55]. More nanosilver in vitro and in vivo toxicity studies have been performed in mammalian species have shown that silver nanoparticles have the capability to enter cells and cause cellular damage [56, 57]. Adverse effects of nanoparticles on human

health depend on individual factors such as genetics and existing disease, as well as exposure and nanoparticle chemistry, size, shape, agglomeration state, and electromagnetic properties. Researchers have shown that while exposure to nanosilver is toxic under certain experimental conditions, other researchers have shown that nanosilver is non-toxic under similar experimental conditions. This is because, there are difficulties in monitoring nanomaterial behavior when dispersed in physiological solutions as the latter often contain particulate and charged materials that will mask the true size distribution and charge measurements of the nanomaterials themselves.

Thus, due to the lack of reliable nanosilver toxicity data in the literature, the silver nanoparticles are safe now. But scientists have investigated the toxicity of silver nanoparticles as well as try to reduce the toxicity through immobilization of this novel metal.

1.5 Strategies for immobilizing silver nanoparticles onto planar substrates

There are lots techniques that have been established for the immobilization of silver nanoparticles (AgNPs) onto planar surface. Some of them are chemical modification, nanolithography, self-assembly, enzyme immobilization, chemical deposition, physical vapour deposition, pulsed filtered cathodic vacuum arc deposition, sputtering deposition techniques, laser photoreduction, plasma treatment, vacuum uv-activation, Layer-by-layer deposition, microwave-assisted immobilization, sonochemical, mussel-inspired strategy etc. [58-71]

1.5.1 Chemical modification

Immobilization of metal nanoparticles (MNPs) on a solid substrate can be achieved by chemical modification of that surface. The processes by which solid substrates are chemically modified prior to immobilization of nanoparticles are namely silanization, the sol-gel process, and use of bifunctional compounds.

1.5.1.1 Silanization

Silanization of surfaces is a common strategy for immobilizing MNPs. In general terms, silanization involves covering the silanol groups of a surface with alkoxysilane compounds, and is believed to occur by a stepwise reaction. It includes rapid hydrolysis of alkoxy groups with liberation of silanol groups and release of alcohols and formation of siloxane linkages on

the surface through covalent bonds between silanol groups, accompanied by the release of water molecules. Thermal curing of the silanized substrate is common in order to limit the heterogeneous multilayer formation produced by polymerization at the free silanol groups. Selection of the most appropriate organofunctional silane compound is important for the MNPs required to be attached to the surface. Both thiol-terminated and amine-terminated alkoxysilane compounds are commonly used for the silanization when immobilization of gold and silver NPs (AuNPs and AgNPs) is intended. Different functional organosilane compounds, including 3-mercaptopropyltrimethoxysilane (MPTMS), 3-mercaptopropyltrimethoxysilane (MPTES), 3-aminopropyltrimethoxysilane (APTMS), 3-aminopropyltriethoxysilane (APTES), N-[3- (trimethoxysilyl)propyl]ethylenediamine (TMSPED), have been used for the deposition of MNPs on a broad variety of surfaces. The adhesion of negative, citrate capped MNPs to thiol-terminated substrates and their stability have been shown to be at least four times greater than the electrostatic interactions with amine-terminated substrates.

1.5.1.2 Sol-gel technique

The sol-gel technique is an efficient technique for surface modification that provides high stability and high surface area. The selection of the experimental conditions used for the sol-gel process defines the properties of the sol-gel-derived material. There are two main reactions involved in the sol-gel production are hydrolysis of the precursor under acidic or alkaline Conditions and polycondensation of the hydrolyzed products. A polymeric network is produced on a solid surface by means of sol-gel process and the subsequent immobilization of metallic nanomaterials happen.

1.5.1.3 Use of bifunctional compounds

Bifunctional compounds can also be used for the attachment of MNPs, and the efficiency of chemical modification by using bifunctional compounds depends on the nature of both the MNP and the solid substrate. The idea is to anchor the molecule to the surface by one of its functional groups, leaving the other functional group free to bind the nanoparticles. For example, the modification of the glass surface with AgNPs is done by derivatized silanes, such as aminopropyltrimethoxisilane (APTMS) or mercaptopropyltrimethoxysilane (MPTMS) [59]. Here, bifunctional compounds behave as molecular linkers between MNPs and the corresponding surface.

1.5.3 Lithographic technique

In the lithographic technique, photolithography and nanolithography are widely used techniques. The most used modern nanolithographic techniques are focused ion beam (FIB) milling and electron-beam lithography (EBL). These techniques allow the fine control over the size and shape of the nanostructures. FIB and EBL have been developed to obtain both nanovoids in continuous metallic thin-films or MNPs in solid supports with very controllable optical properties. The electron-beam lithography technique consists of a 10–50 keV beam of electrons focused on a solid support and generally electronresistcovered SiOx/Si wafers are used in this purpose. As in regular photolithography, EBL can be performed using either positive or negative resists. The electron beam selectively etches off regions of the positive resist from the surface in a preset form. In contrast, the region exposed remains if a negative resistor is used. The most used electronresist is poly (methyl methacrylate) (PMMA) which is a positive EBL resistor. Using the method, circular and triangular silver nanoparticles of 200nm diameter were successfully immobilized in to solid substrate for surface-enhanced Raman scattering (SERS) performance.

1.5.4 Self-assembly method

The self-assembly method may be two types such as direct-assembly and in situ assembly. The assembly may be direct to immobilized AgNPs into unsmooth surface like paper. AgNPs are usually synthesized by citrate reduction of aqueous silver salt solutions and then assembled onto paper. The citrate groups serve the dual role of reducing agent and stabilizer. They impart negative surface charges to nanoparticles from weakly bound citrate ions, which prevent agglomeration in solution and immobilize them on the substrates through electrostatic self-assembly. In situ assembly is initiated by attaching metal ions onto cellulose surfaces through a physisorption process that involves electrostatic interactions and followed by reduction of metal ion to zero-valence metal nanoparticles [72]. Compared to the direct assembly method, in situ assembly does not require protective citrate ions. The absence of citrate ions can be helpful in applications such as catalysis, since their surface coverage may reduce the reactivity of the nanoparticles.

1.5.5 Enzyme immobilization method

Enzyme immobilization method utilizes the interaction between amino and cysteine groups of proteins with nanoparticles which is as strong as that of the commonly used thiols. Cholesterol oxidase, Haloalkane dehalogenase, Laccase, Keratinase, α-Amylase, β Galactosidase, Lipase, Glucose oxidase, α-Chymotrypsin, Diastase, urease etc. are reported enzyme that have used in the immobilization of different nanomaterials. A typical biosensor was made using urease to immobilized AgNPs [73].

1.5.6 Plasma treatment method

The plasma treatment method is found effective in the modification of textile surface. In the plasma treatment method, the solid substrate such as cotton fibre is treated with plasma species such as oxygen species to obtain variety of oxygen functional groups C–O, C=O,–O–C=O, –COH, –COOH on the fabric through the reaction between the active species induced by the plasma in the gas phase and the C-surface atoms. These functional groups are further used for immobilization of AgNPs after reduction of silver ions.

1.5.7 Microwave-assisted immobilization

The microwave-assisted immobilization is the most recently developed method for immobilizing silver nanoparticles onto solid substrate. In this method, the solid substrate, reducing agent, silver salt solution are taken into a reaction vessel and exposed to microwave radiation with GHz frequency for the immobilization of nanoparticles. Like microwave method, sonochemical is another approach for the immobilization of silver nanoparticles in to solid substrate.

1.5.8 Bio-inspired strategy

The most popular and acceptable technique for immobilization of silver nanoparticles that have reported is mussel-inspired strategy. Herein, Dopamine and other catechol compounds have used as coating materials for numerous substrates. Dopamine, also known as 3,4-dihydroxy-phenylalanine, is an important constituent of mussel-secreted proteins for attachment to wet surfaces. The catechol groups in dopamine has superb reducing and adhesion properties which enables strong adherence to a wide range of materials and this makes dopamine a potentially outstanding anchor for various modifications to surface

chemistry. In addition, it has reported that the self-polymerisation of dopamine gives a thin layer film on different substrates which has excellent reducing and adhesion properties for immobilization of nanoparticles. This makes the strategy versatile because it requires simple ingredients and mild reaction conditions and it is applicable to multiple materials with complex shapes. Unlike other structures, that of dopamine forms a robust interface between Ag NPs and organic molecules. It can strongly coordinate with metal substrates through two adjacent hydroxyls [74].

1.6 Research goals and organization

In this research we used a novel mussel-inspired approach to immobilized silver nanoparticles towards the synthesis of antibacterial paper. With the increased requirement of the paper based antibacterial products associated with nanoparticles, our research is another approach for the development of silver nanoparticles based antibacterial paper. Herein, we chemically modified the paper surface by incorporating succinic group initially. Afterward, the use of coupling reaction between succinic group and -NH_2 group of dopamine chemically attached the dopamine molecules onto paper surface. Using the reducing and adhesion properties of catechol groups of dopamine the silver ions is successfully reduced into silver nanoparticles and set them onto paper surface. This method is very facile, robust and rapid as it just required the immersing of dopamine coated paper in silver salt solution. The silver nanoparticles deposited paper thus obtained is characterized by x-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), scanning electron microscopy (SEM), Fourier transform infrared (FTIR), analyses. The effect of time on the deposition silver nanoparticles onto paper surface is also investigated using SEM and thermal analysis technique. The prepared paper sample is also tested for antibacterial activity. The possible outcome of our research is to establish a new facile route for the immobilization of silver nanoparticles onto paper matrix which gives a robust and adhesive paper surface that will tether the silver nanoparticles strongly and thus gives us an top quality antimicrobial paper that would kill bacteria and might be useful for hand hygiene, food wrapping and storing.

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Chapter 2
Experimental

2.1 Materials and Instruments

2.1.1 Chemical and reagents

The chemicals and reagents used in this work were analytical grade and used without further purification. De-ionized water was used as solvent to prepare most of the solutions of this work. The chemicals and reagents used in this work are listed below:

(i) Ethanol (absolute) (Merck, India)
(ii) Toluene(E. Merck, Germany)
(iii) Acetone (analytical grade)
(iv) Bromocresol green indicator (analytical grade)
(v) N-(3-dimethylaminopropyl)-N‟-ethylcarbodiimide hydrochloride (Sigma-Aldich)
(vi) Succinic anhydride (Loba, India)
(vii) Disodium hydrogen phosphate(Merck, India)
(viii) Potassium dihydrogen phosphate
(ix) Potassium chloride (reagent grade)
(x) Sodium chloride (reagent grade)
(xi) Silver nitrate (analytical grade)
(xii) Dopamine hydrochloride (Sigma-Aldich)
(xiii) Hydrochloric acid (analytical grade)
(xiv) Sodium hydroxide(E. Merck, Germany)
(xv) Pyridine (Loba, India)

2.1.2 Instruments

Analysis of the samples was performed using the following instruments:

(i) Fourier Transform Infrared Spectrophotometer (SHIMADZU FTIR-8400)
(ii) Field Emission Scanning Electron Microscopy (JSM-7600F, Tokyo, Japan)
(iii) UV-visible Spectrophotometer (Shimadzu-1800)
(iv) Furnace (Naber Therm, N-7, Germany)
(v) pH meter (Hanna, HI 8424, Romania)
(vi) Digital Balance (AB 265/S/SACT METTLER, Toleto, Switzerland)

2.2 Method of preparation

2.2.1 Cellulose paper Pretreatment

Whatman No.5 filter paper was cut into round pieces with a hole-puncher or using scissor featuring a diameter of close to 3.5 cm. 0.5g of cutting paper was immersed in an aqueous solution of 10wt% NaOH (150mL) for 18h. The swollen samples were repeatedly washed with absolute ethanol until a neutral solution was obtained. The washing absolute ethanol was checked using pH paper. The paper sheets were used for further reactions after the sogginess of the paper was removed by air. 50mg of pretreated cellulose (WhatmanNo.5) contains 0.91mmol of active hydroxyl groups [1].

2.2.2 Succinylation of cellulose paper

Pretreated cellulose paper (0.5g, 9.1mmol of active OH-groups) was converted into succinylated cellulose paper containing free carboxylic groups by reacting with succinic anhydride in a mixture of pyridine and toluene. The succinic anhydride (2.5g, 25mmol) was dissolved in pyridine (10ml) and then toluene (35ml) was added to the mixture. Afterward, the pretreated cellulose paper was added into the resulting mixture and heated at 90°C for 17h. After the heat treatment the paper was got out from the reacting solution and washed with toluene at least 2 times using shaker and then repeatedly with acetone at least 5 times and dried in air before coupling reaction. After drying at 60°C in an oven for 1 h and in a desiccator overnight the mass percent gain (mpg) was calculated.

2.2.3 In situ reduction process and immobilization of silver nanoparticles

Dopamine molecules was added into Succinylated paper by coupling with -NH_2 group of dopamine and free –COOH group of succinylated paper in presence of coupling agent EDC (N-3-Dimethylaminopropyl-N′-ethylcarbodiimide). About 7ml of PBS buffer (pH 5.0-5.5), 0.06 mmol of dopamine hydrochloride, 0.1 mmol of N-3-Dimethylaminopropyl-N′-ethylcarbodiimide hydrochloride was taken into a beaker and the pH was maintained at pH= 5.0 by adding 0.1M HCl/NaOH. To this mixture, previously prepared succinylated paper was added and the mixture was stirred gently for 24h at a shaker. Afterward, the paper was washed with PBS buffer at least 2 times and then with deionised water at least 5 times. After washing, the paper was dried in air about one hour and added to the Silver ammonia solution prepared by adding ammonia aqueous solution (2 wt %) into 5 mg mL^{-1} $AgNO_3$ solution

until brown precipitation was just dissolved at room temperature. After the completion of the required time, the Silver ammonia solution was collected for further investigation and the paper was washed with deionised water using a shaker at 150 rpm until the washing liquid appears clear. In every case of different contact time between ammoniacal silver solution and dopamine added paper we used same concentration of silver nitrate solution

2.3 Sample characterization

2.3.1 Degree of substitution

The degree of substitution of succinylated paper was determined by back titration method [2]. 0.2 g of the sample was immersed in 100ml of an aqueous 0.02M $NaHCO_3$ solution and the resulting mixture was stirred for 2 h at room temperature. After filtration, the excess of $NaHCO_3$ was back-titrated with 0.02M HCl using methyl orange as the indicator. The titration was repeated three times and the average value of the HCl volume was used for the calculations.

The DS was calculated by using the following equation:

$$DS = \frac{162 \times n_{COOH}}{m - 100 \times n_{COOH}} \quad \cdots \quad \cdots \quad (1)$$

Where, 162 $gmol^{-1}$ is the molar mass of an Arbitrary Glucose Unit (AGU), 100 $gmol^{-1}$ is the net increase in the mass of an AGU for each substituted succinyl group, m is the weight of the sample analyzed and n_{COOH} is the amount of COOH calculated from the obtained value of the equivalent volume of known HCl molarity according to the following

Equation:

$$n_{COOH} = V_{NaHCO_3} \times C_{NaHCO_3} - V_{HCl} \times C_{HCl} \quad \cdots \quad \cdots \quad (2)$$

2.3.2 Mass percent gain

The mass percent gain (mpg) was calculated according to

$$\text{mpg}\ (\%) = \left(\frac{m_{\text{modified}} - m_{\text{unmodified}}}{m_{\text{unmodified}}}\right) \times 100 \qquad \cdots \qquad \cdots \quad (3)$$

where, m denotes the mass of the modified and unmodified cellulose.

2.3.3 FTIR analyses

The infrared spectra of the dried reference, succinylated, dopamine and silver added paper samples were recorded on an FTIR spectrometer in the region of 4000 – 400 cm^{-1}. Since, paper is a collection of long chain cellulose polymers and it has strong H-bond between them, it is not easy to make powder of it. First the paper was cut as much as possible small sized pieces and then grinded into small powder. IR spectra of the solid samples were frequently obtained by mixing and grinding a small amount of materials with dry and pure KBr crystals. The mixing and grinding were done in a mortar by a pestle. The powder mixture was then compressed in a metal holder under a pressure of 8–10 tons to make a pellet. The pellet was then placed in the path of IR beam for measurements.

2.3.4 Scanning Electron Microscopy (SEM)

The surface morphology of the synthesized AgNP-papers of different contact time with ammoniacal silver solution (i.e. 5 min., 15 min., 4 h, 8 h, 12 h, 18 h) and unmodified reference paper was adopted using Field Emission Scanning Electron Microscopy (FE-SEM). The completely air dried paper samples were glued on a conducting carbon strip. The sample loaded strip was then mounted to a chamber that evacuated to ~ 10^{-3} to 10^{-4} torr and then a very thin platinum layer (~few nanometers thick) were sputtered on the sample to ensure the conductivity of the sample surface. The sample was then placed in the main SEM chamber to view its surface. The microscope was operated at an accelerating voltage of 5.0 kV. The system was computer interfaced and thus provides recording of the surface images in the computer file for its use as hard copy.

2.3.5 X-ray photoelectron spectroscopy (XPS)

XPS measurements were performed on samples that had been prepared within 4 days using the AXIS ULTRA spectrometer (Kratos Analytical). The base pressure in the analytical chamber was <3 $x10^{-8}$ Pa. The monochromatic AlKα source (hν=1486.6 eV) was used at a power of 210 W. The photoelectron exit angle was 90^0, and the incident angle was 35.3^o from the plane of the surface. The analysis spot was 400 x 700 μm. The resolution of the instrument was 0.55 eV for Ag 3d peaks. Survey scans were collected for binding energies from 1100 to 0 eV with an analyzer pass energy of 160 eV and a step of 0.35 eV. The high-resolution spectra were run with a pass-energy of 20 eV and a step of 0.1 eV. Relative sensitivity factors (RSFs) for different elements were as follows: 1 for F (1s), 0.477 for N (1s), 0.955 for Br (3d). Only one set of XPS scans was performed on a given sample; therefore, XPS analysis before and after surface reactions were performed on different samples.

2.3.6 Atomic force microscopy (AFM)

AFM experiments were performed on samples of unmodified, dopamine added paper and two AgNP-paper produced by fixing 15 min and 12 h contact time between ammoniacal silver solution and dopamine added paper. The experiments were done using a MFP-3D (Asylum) operated in tapping mode with commercially available Si cantilevers (Olympus, 300 kHz frequency). The force constant of the cantilevers was 42 N/m. The oscillation frequency used for tapping mode was 310 ± 5 kHz.

2.3.7 Microbial experimentation

In virto antimicrobial activities of silver nanoparticles immobilized paper (AgNP-paper) discs on fish and shrimp pathogenic bacteria were determined by disc diffusion method [3]. For qualitative measurement of microbial activity, about 6 mm diameter AgNP-paper discs were prepared by the above mention method and four samples (i.e. 15min, 8h, 12h, 18h) were prepared varying the contact time of ammonical silver solution with dopamine added paper. The antimicrobial activity was tested taking thirty microliter of fresh bacterial culture and spread on the Iso sensei Test Agar plates (Micro Master, India). Then, the sterilized (autoclaved and dried) AgNP-paper discs were aseptically placed on the culture plate and incubated at 28^oC for 24h in an incubator The plates were examined for possible clear zones after incubation at 28^0C for 24 hours. The presence of any clear zone that formed on the

surface of the AgNP-paper was recorded as an indication of inhibition against the microbial species. The diameter of AgNP-paper discs and diameter of zone of inhibitions were measured and the ratios between the diameters were calculated.

2.3.8 Antifungal sensitivity test

For antifungal test we had prepared silver nanoparticles immobilized paper (AgNP-paper) discs of diameter 6mm using the same procedure as mention above. Herein, we exploit two AgNP-paper samples fixing 8h and 18h contact time between the dopamine added papers with ammoniacal silver solution. The culture of fungal strain (i.e. Magnaporthe oryzae) was done using 12 mL of potato dextrose agar on potato dextrose agar (PDA) plates after 14–21 days of inoculation [4].Then, the sterilized (autoclaved and dried) AgNP-paper discs were aseptically placed on the culture plate and incubated in dark at 28^{o}C for 7 days in an incubator. The plates were examined for possible clear zones after incubation.

2.3.9 Toxicity study

Probable toxicity of the silver nanoparticles immobilized paper (AgNP-paper) was found out using phytotoxicity test. The 6mm diameter AgNP-paper discs were prepared in the same method as talk about earlier. . Herein, we exploit two AgNP-paper samples fixing 8h and 18h contact time between the dopamine added papers with ammoniacal silver solution. The tests were applied in rice and barli leaf and wheat seed. The seed germination was carried out putting the sterilized (autoclaved and dried) AgNP-paper discs aseptically in the culture media setting 95% humidity at 28^{o}C and applying 16h light and 6h dark. For leaf, the sterilized AgNP-paper discs were placed on the leaf using sterile forceps and examined for the possible appearance of dark circle around the AgNP-paper discs after 7 days.

2.3.10 Silver deposition study

In this study we use ash-less filter paper. About 0.1g paper was taken for deposition of each different contact time with ammonia silver solution. The paper was chemically modified as same described in earlier. The deposition was performed for 15min, 4h, 8h, 12h and 18h. After the deposition all the samples were dried in air at least one week and then weight. Then each of the samples was put into a Crucible and burnt into a furnace at 700^{0}C for 2 hours. Afterward, the Crucibles were cooled putting them into a Desiccator for one day and weight. The same process was followed for blank measurement.

2.3.11 Ultraviolet – visible study

The ultraviolet-visible spectral analysis of the ammoniacal silver solutions of different time (i.e. o min., 15min., 4 h, 8 h, 12 h) after the completion reaction with dopamine added paper was carried out a double beam spectrophotometer. The existence of silver nanoparticles in the ammoniacal silver solution after reaction was particularly studied using this technique. All the solutions were first diluted with DI water and then the absorbance was determined spectroscopically.

2.3.12 Silver release study

The silver release studies were performed in a 200 ml batch reactor using deionised water (DI) as solvent (Fig. 4.1). For that 2x2 cm^2 air dry silver deposited paper with desire time of contact with silver solution was taken and then sonicated with 50 ml DI water at about 5 minutes. After the sonication the paper was dry 1 day at 60^0C and then used for silver release study. The paper was set and dipped into deionised water (DI) as shown in Fig. 4.1. A specific volume (20 ml) of sample (DI) was taken out at desired intervals with a syringe and was analyzed after acid digestion. This was followed by appropriate dilution of the test samples for the measurement of silver release using atomic adsorption spectroscopy (AAS).

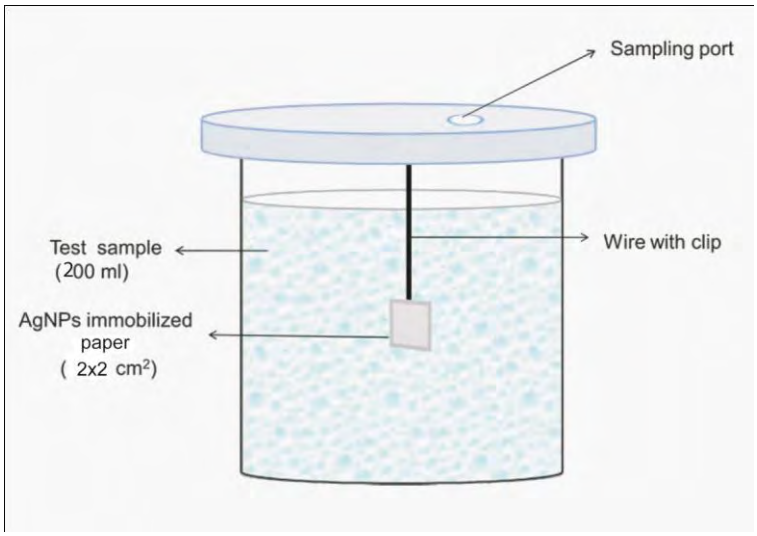

Fig 2.1 200 ml batch reactor for studying the silver ion release into DI water

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CHAPTER 3
RESULTS
AND
DISCUSSION

3.1 Synthesis of antbacterial paper

Cellulose is a long chain, linear, natural polymer where the repeating unit is D-anhydroglucose unit. The repeating units are composed together by β-1, 4 linkages to form the long chain [1]. In this way it shows a high degree of crystallinity in its native state. Moreover, the presence of –OH groups in glucose unit form intermolecular H-bond between adjacent cellulose chains. Therefore, cellulose is not very reactive cause all the -OH group of cellulose chain are not available for reaction. In order to increase the availability of –OH group of cellulose chain it needs pretreatment. However, in this study we use filter paper as a starting material for chemical modification. As filter paper is a cellulosic material it also needs pretreatment to increase the availability of OH groups of cellulose chain. The use of 10% aqueous solution of NaOH for pretreatment disrupts the extensive H-bond between cellulose fibers and could deprotonate the primary OH group of glucose unit [2]. Thus, the pretreatment increases the accessicibily of the filter paper so that the reagents could easily penetrate inside the cellulose substrate. The use of bromocresol green indicator solution also gives excellent detection colour between treated and untreated paper (Fig. 3.1). After the treatment the use of absolute alcohol for washing the paper remove alkali and prevent further formation of H-bond. However, the chemical reaction by succinic anhydride with untreated and pretreated paper to introduce –COOH group was performed in toluene and pyridine solvent at 90°C within 17h. This gives the concentration of carboxylic functions 4.14 and 6.13 mmol/g calculated by eq. (2) and the corresponding DS value 1.1 and 2.56 calculated by eq. (1) written in experimental section. The mass gain after succinylation was found 1.50% and 4.5 % calculated by eq. (3) for untreated and pretreated paper respectively. These also demonstrate that the pretreatment increases accessibility or reactivity of -OH group of the cellulose fiber. The colour of the untreated and pretreated succinylated paper in that case after dipping and then drying with indicator solution also shows different intensity of yellow color (Fig. 3.1). The coupling reaction between the -COOH group of succinylated paper and -NH_2 group of dopamine was done in presence of EDC at a room temperature within 24h. After the reaction the paper was remained identical as the starting material. This suggested that the coupling reaction happen successively instead of the polymerization of dopamine molecules. The colour of the paper after dipping and drying with indicator gives greenish-yellow colour which also indicates the decrease of carboxylic acid functional groups. The air dry dopamine added paper when added into ammoniacal silver nitrate solution the paper changes its colour

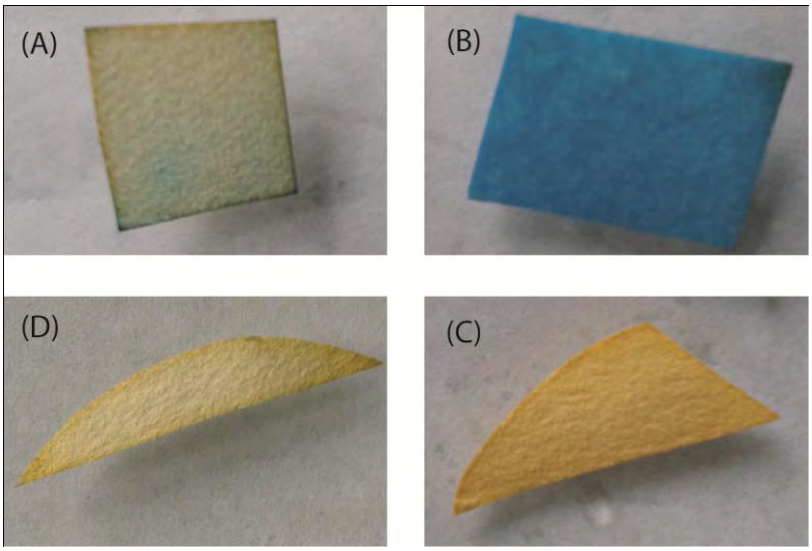

Fig. 3.1 Optical images of colour change after dipping and drying with bromocresol green indicator solution. (A) Reference (B) After deprotonation (C) After succinylation (D) After dopamine addition

from white to brown within 1 min indicating the deposition of AgNPs to the paper. Herein, for the first time we use coupling reaction to conjugate dopamine molecules into paper matrix followed by immobilization of AgNPs using the adhesion and reduction properties of catechol groups of dopamine. This not only immobilizes the AgNPs but also attach it into the paper matrix with great robust wherever the coupling reactions occur. Moreover, our method retain the properties of the paper such as flexibility, mechanical strength etc. and thus make the method facile and cost-effective. Fig. 3.2 shows the overall Schematic working procedure of our project.

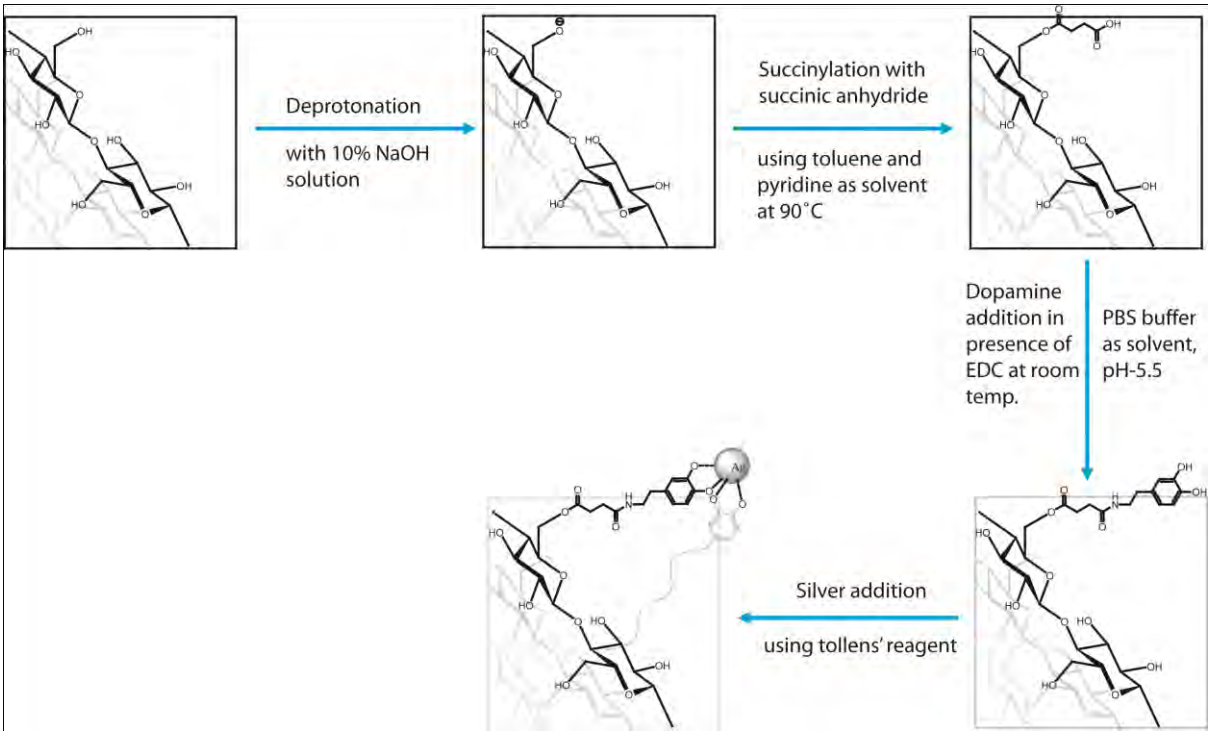

Fig. 3.2 Schematic representation of the synthetic process and optical image of silver added paper.

3.2 Functional group analysis using Fourier Transform Infrared spectroscopy (FTIR)

In order to make sure the functional groups were conjugated into the paper matrix after chemical treatment, the chemically modified paper was first characterized by FTIR spectroscopy. Fig. 3.3 shows the FTIR spectra of ungrafted and grafted papers i.e. from starting to final modification. The first one is the normal cellulose based paper. Where the peak at 1633 cm^{-1} is for water adsorption from air by cellulose materials [3] and other characteristic peak of cellulose materials that are found in our study are shown in table 3.1.

Table 3.1: Characteristic peak and interpretations correspond to cellulose paper.

Wave number (cm^{-1})	Interpretations
3440.2	-OH stretching
2902	C-H stretching
1428.3	$-CH_2$ bending
1371.4	C-H bending
1166	-OH bending, C-O stretching
1059	C-O stretching
896.9	$-CH_2$ bending

The 2nd one is for succinylated paper where the peak appears at 1740 cm^{-1} which is the characteristic peak of carbonyl bond of carboxylic acid groups. The increment of the peaks in the spectra at 2895 cm^{-1} and 3340 cm^{-1} corresponds to asymmetric stretching $-CH_2$ and stretching of -OH also indicate the introduction of succinic group. The 3rd one is for dopamine added paper where the peak at 1740 cm^{-1} again rises indicating the formation of amide bond alone with >C=O bond. There is a reasonable increase of peak at 3340 cm^{-1} and 1740 cm^{-1} representing the formation of amide groups and the increase of -OH groups of the paper matrix as one dopamine molecule has two hydroxyl group (catechol group). The last one is for silver added paper. The arising of peaks at 1555 cm^{-1} is for the production of catechol ring between the silver nanoparticles and catechol groups.

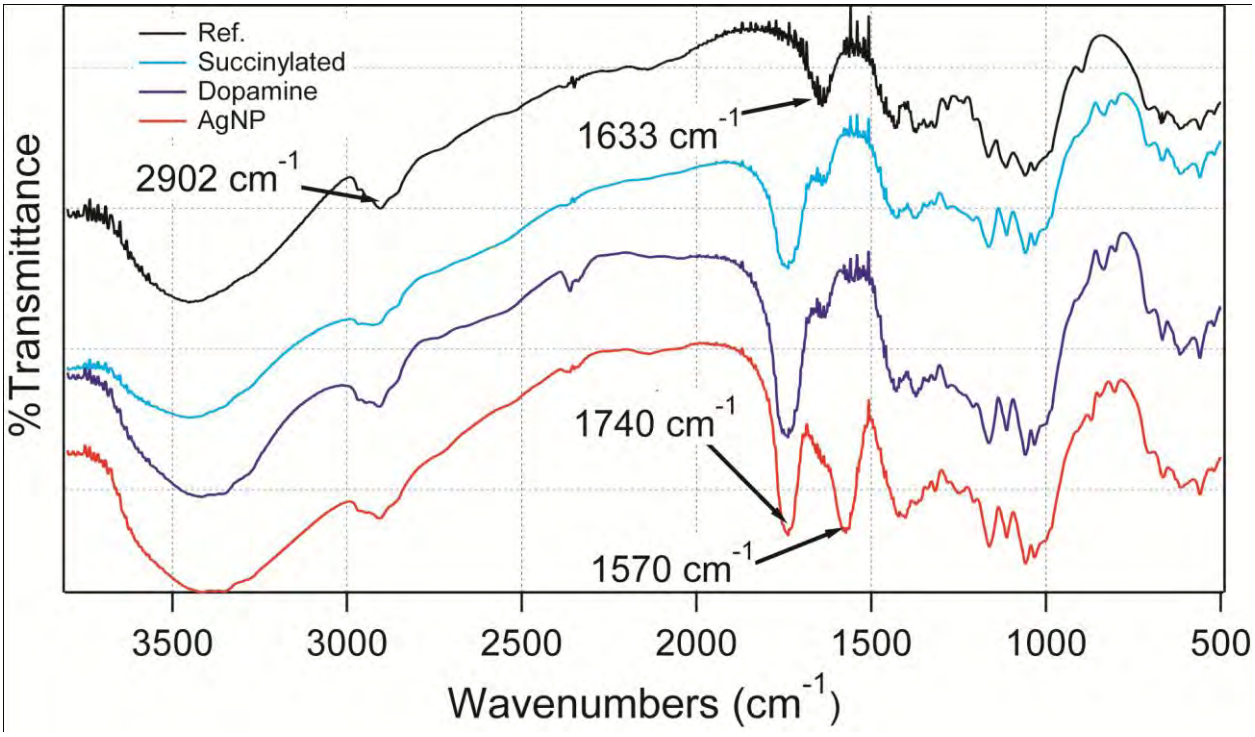

Fig. 3.3 FTIR spectra of reference, succinylated, dopamine and silver added papers.

3.3 Surface morphology study using Atomic force microscopy (AFM)

In order to get three dimensional images with high resolution, the nanosilver deposited papers with different time of deposition along with reference and dopamine added paper were characterized by atomic force microscopy.

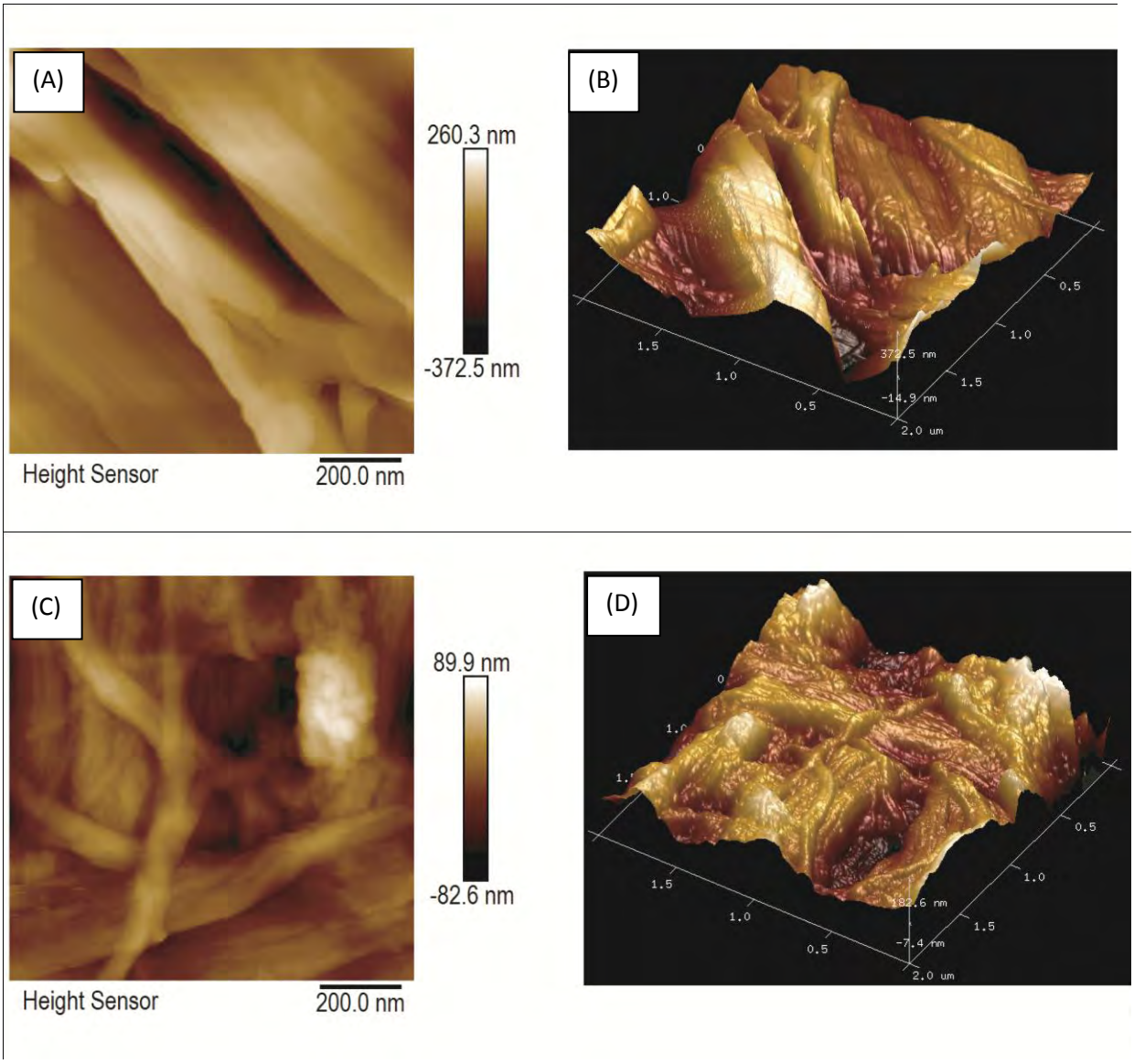

Fig. 3.4 AFM images of reference (A, B) and dopamine added (C, D) paper.

Although paper surface is seen to be smooth in open eye but the surface is not smooth because it is made of collecting the threads of cellulose polymer pressing with high pressure. That is why it has some ups and downs at its surface which are clearly seen from the AFM Fig.ure of reference paper (Fig. 3.4). After the various treatments i.e. the deprotonation, succinylation and finally coupling reaction, there is a very slight change in surface roughness

that is appeared at the surface of the modified paper. This is happened because, the treatment with 10% NaOH solution increases the accessibility of primary hydroxyl group and afterward succinylation add carboxylic acid group onto the surface and further dopamine conjugation reaction add another functional group into the modified surface (Fig. 3.4).

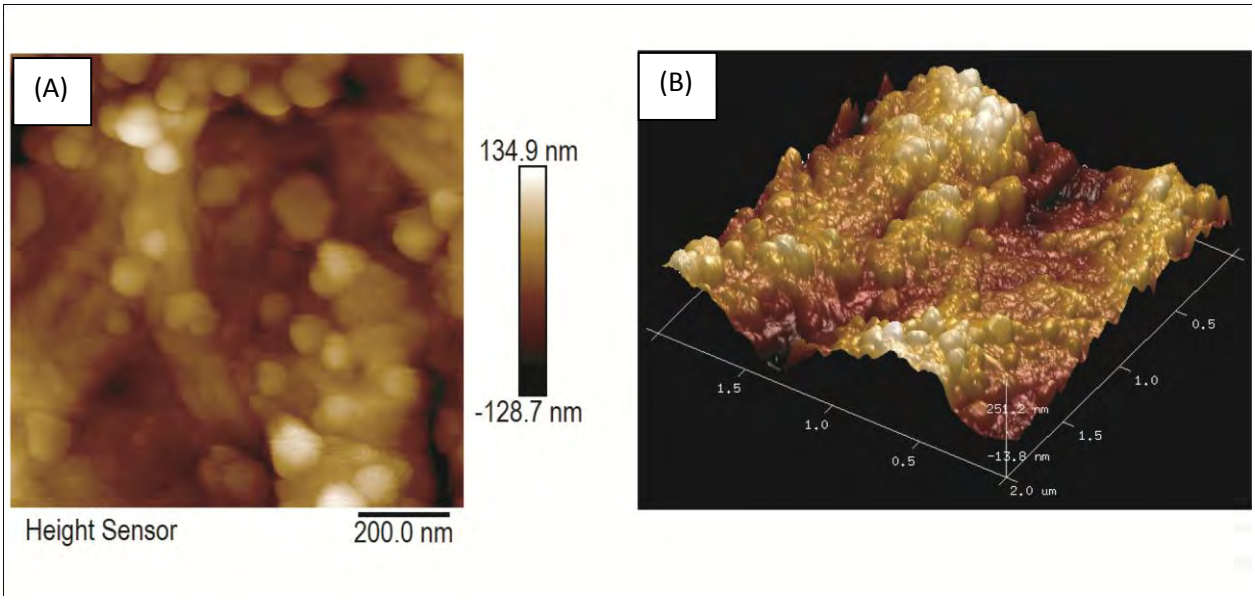

Fig. 3.5 AFM images of nanosilver deposited papers of 15 min. contact time with ammoniacal silver solution (A, B).

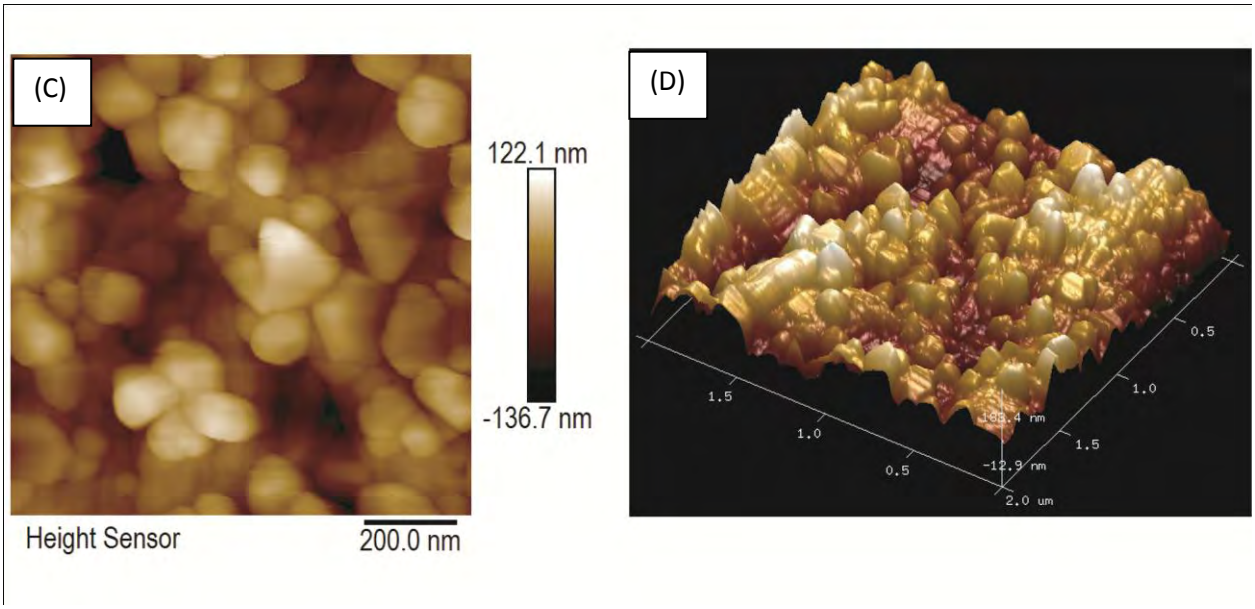

Fig. 3.6 AFM images of nanosilver deposited papers of 12 h contact time with ammoniacal silver solution (C, D).

When the dopamine grafted paper is immerged into ammoniacal silver nitrate solution it turns brown colour from white within 1 minute. Fig. 3.5-3.6 show the two nanosilver deposited paper of different contact time with silver solution that is 15 min. and 12 h. The distinction between the two papers is quite clear. The deposition of silver nanoparticles is quite high in 12 hours than 15 minutes and also the size. This is happened because the silver nanoparticles which are produced by the in situ reduction process become increase their size through aggregation as the time passes and the aggregation is confirmed by FE-SEM image which discussed after this section. Therefore the roughness of 12 hours deposition paper is quite bit of high than the 15 minute deposition. Fig. 3.5-3.6 also show that both the papers contain nanosilver which is fairly spherical in shape.

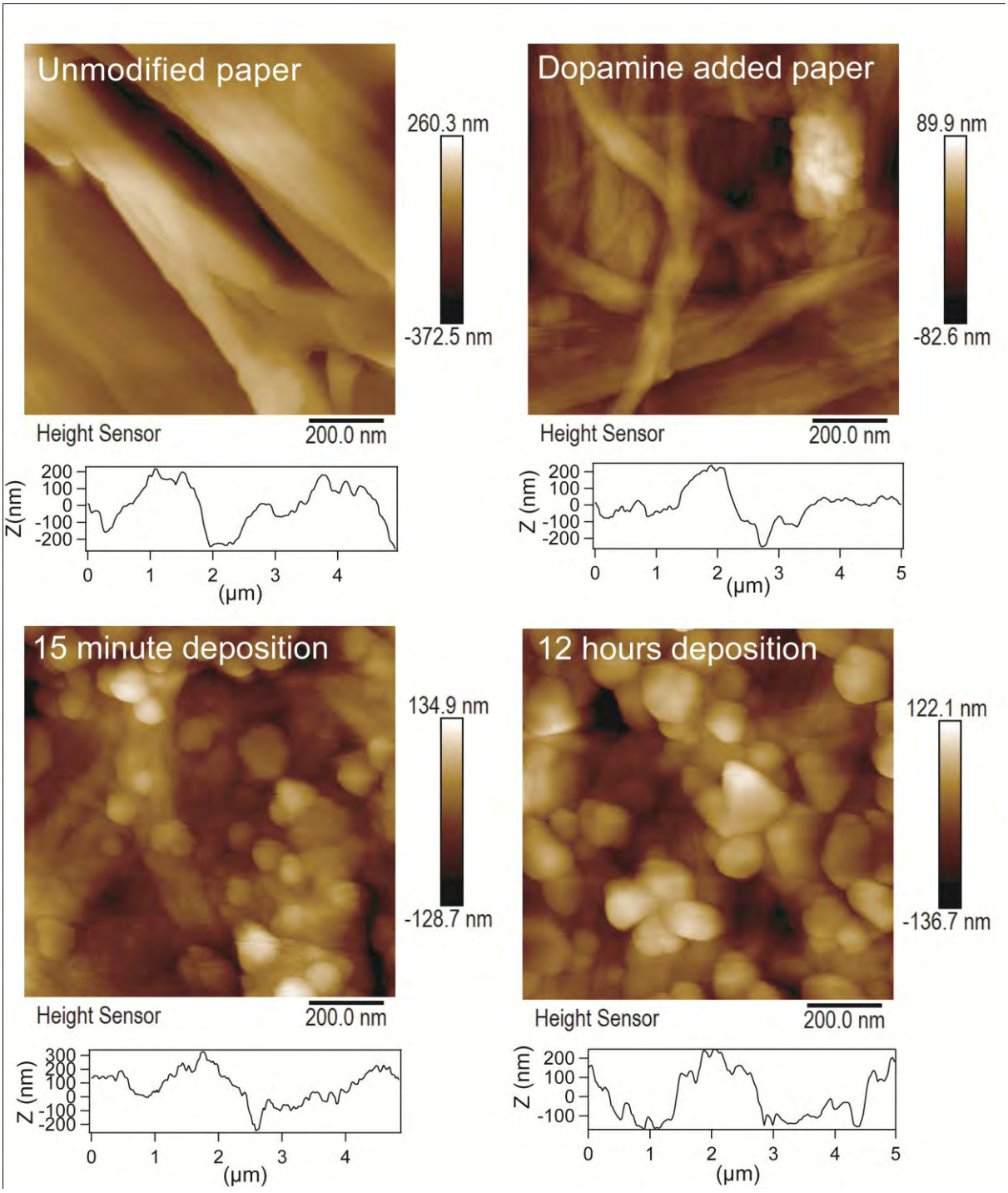

Fig. 3.7 AFM images of reference, dopamine added and nanosilver deposited paper of contact time with ammoniacal silver solution of 15 min. and 12 h with measuring scale.

3.4 Surface morphology study using Scanning electron microscope (SEM)

Surface morphologies of reference and silver nanoparticles (AgNPs) papers of different contact time with ammoniacal silver solution were characterized by virtue of Field Emission Scanning Electron Microscopy (FE-SEM).

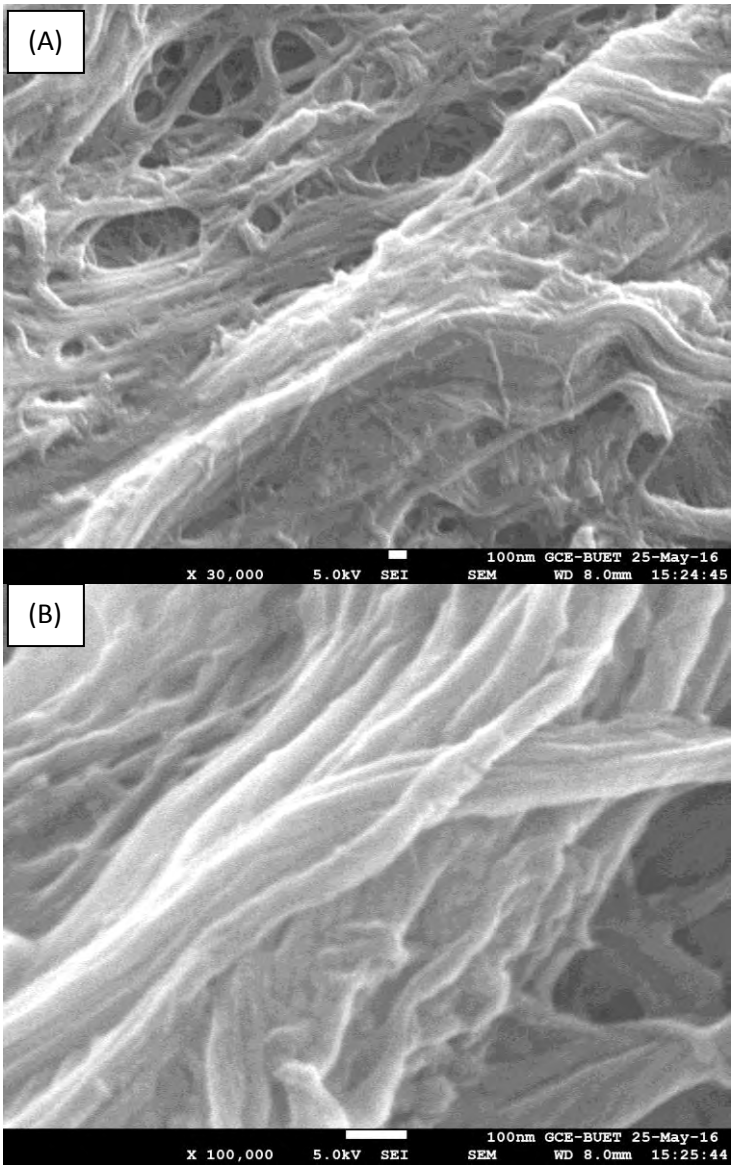

Fig. 3.8 SEM images of reference paper (A) Resolution x30000 (B) Resolution x100000

As paper is made of cellulosic pulp by collecting and pressing them, the surface of the paper is not smooth. The FE-SEM images of reference paper show (Fig. 3.8) the threads gathering

to each other. As it is not possible to arrange them all in line, the paper surface has lots of low depth holes due to random arrangement.

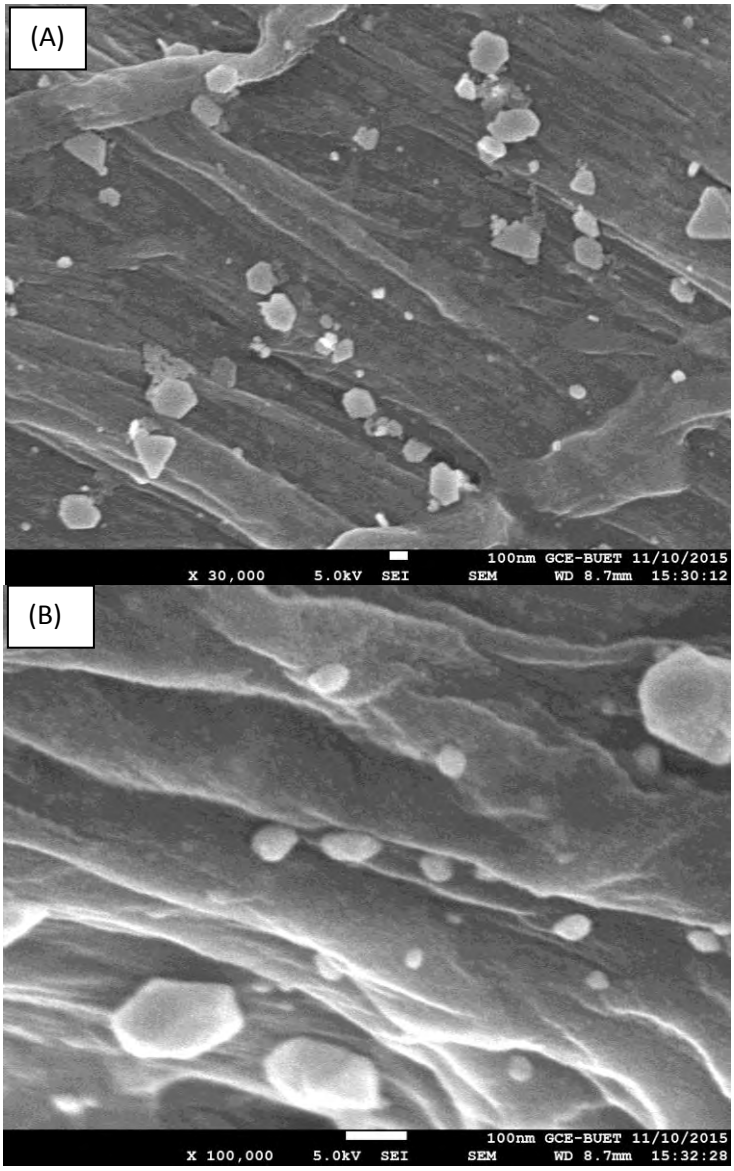

Fig. 3.9 SEM images of AgNP-paper of contact time 5 minutes with Ag^{+} solution
(A) Resolution x30000 (B) Resolution x100000

Fig. 3.9 shows the silver nanoparticles (AgNPs) deposited paper of 5 minutes contact time with ammoniacal silver solution. The deposition is in fact a few and it contains different size

of the particles ranging from 30 to above 100 nm. After passing 15 minutes with silver solution the

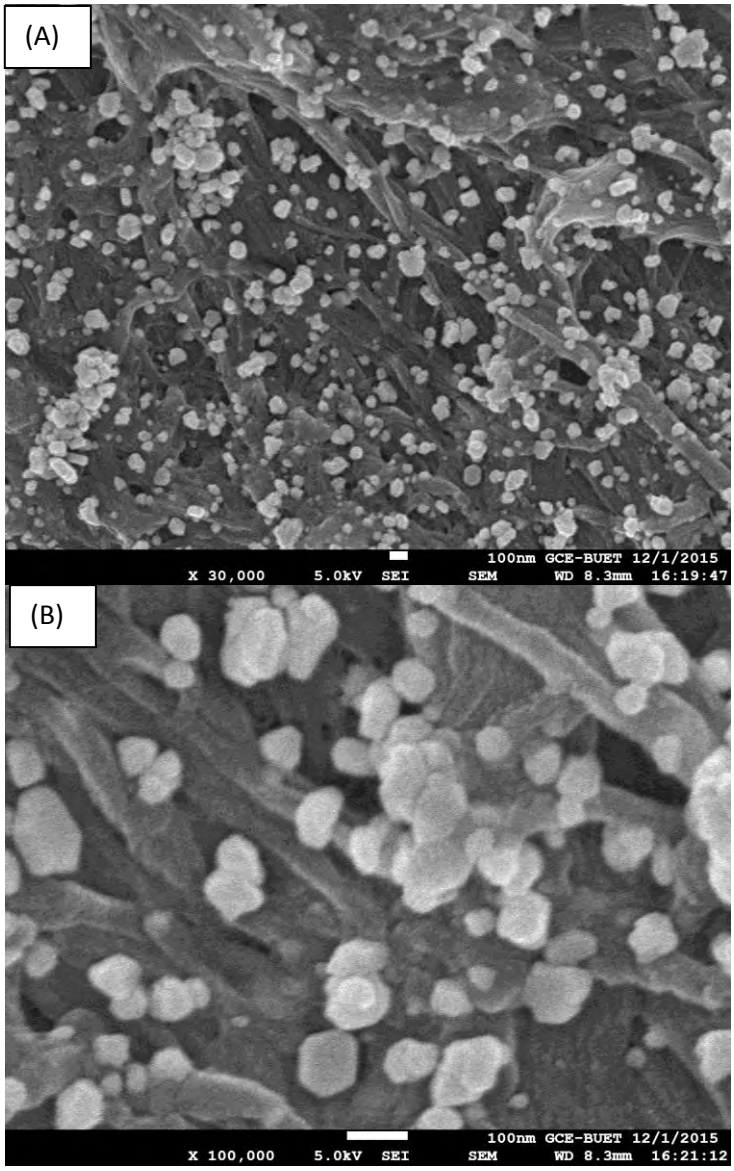

Fig. 3.10 SEM images of AgNP-paper of contact time 15 minutes with Ag^{+} solution
(A) Resolution x30000 (B) Resolution x100000

deposition of AgNPs of paper was also characterized by FE-SEM. Fig. 3.10 shows the 15 minutes deposition paper. From the SEM images it is seen that the deposition is quite uniform all around the paper surface and the size of the particles are about 50 nm to above 100 nm.

After 15 minutes deposition we increase the time gradually. Fig. 3.11 and Fig. 3.12 show AgNPs deposited

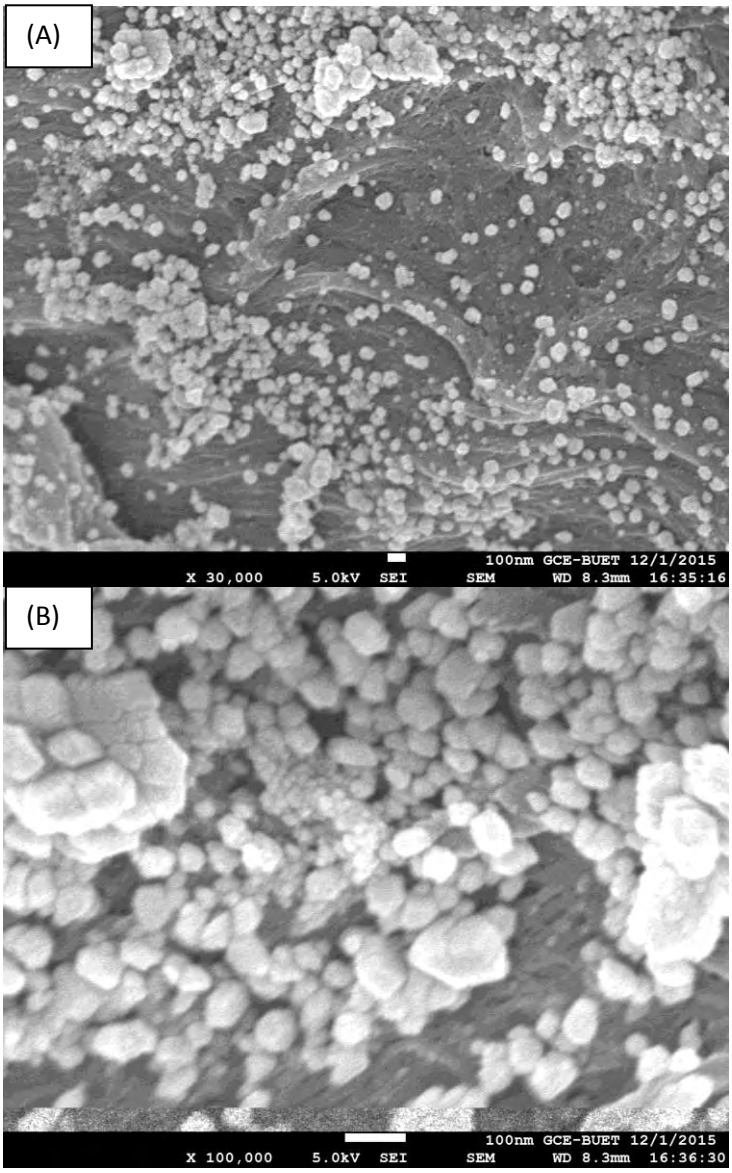

Fig. 3.11 SEM images of AgNP-paper of contact time 4 hours with Ag^{+} solution
(A) Resolution x30000 (B) Resolution x100000

paper of contact time between silver solution and dopamine paper is 4 hours and 8 hours respectively. The two papers have a similarity that in some spots of the surface have clusters

that is totally big and multilayer and some spots are void. Except the clusters, the size of particles is ranging from 50 nm to above 100 nm.

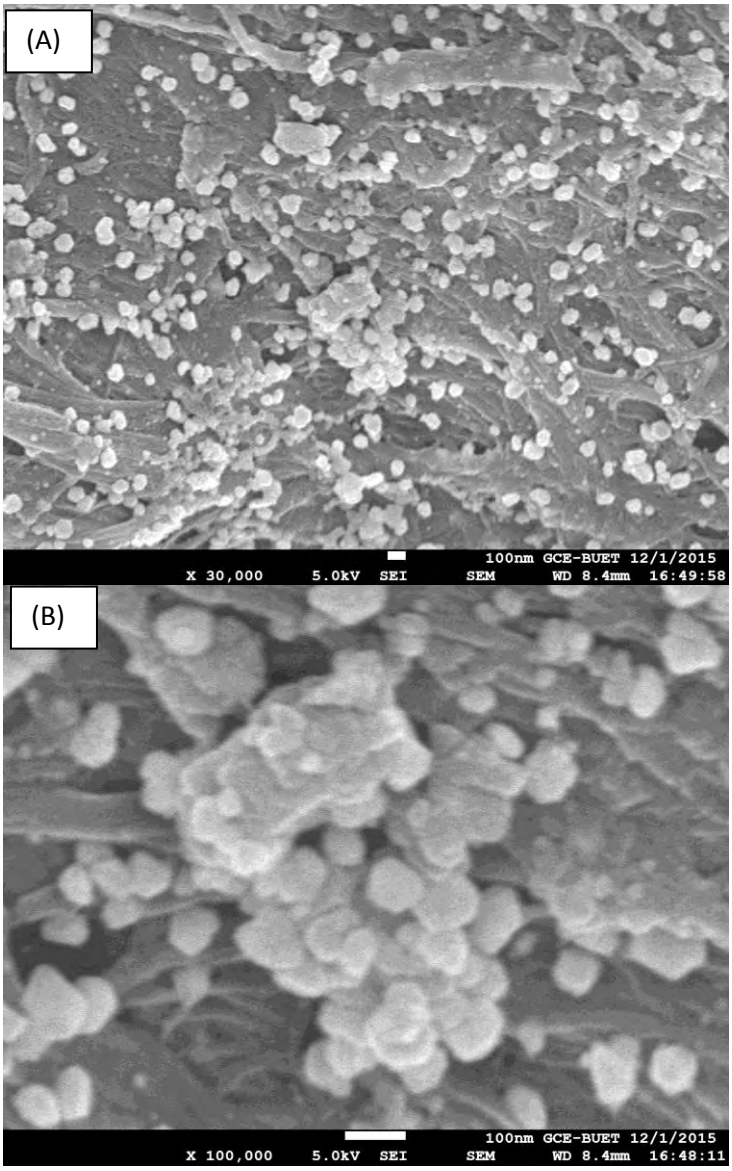

Fig. 3.12 SEM images of AgNP-paper of contact time 8 hours with Ag^{+} solution
(A) Resolution x30000 (B) Resolution x100000

Fig. 3.13 shows 12 hours AgNPs deposition paper which is completely different from other paper. Because the deposition here is absolutely high and we also found (see section 5. table) that, this is the highest deposition of all the other paper in our study. Here we see from Fig.

3.13 that the deposition happen in every section of the paper surface and the size of the particles are also ranging from 50nm to above 100 nm.

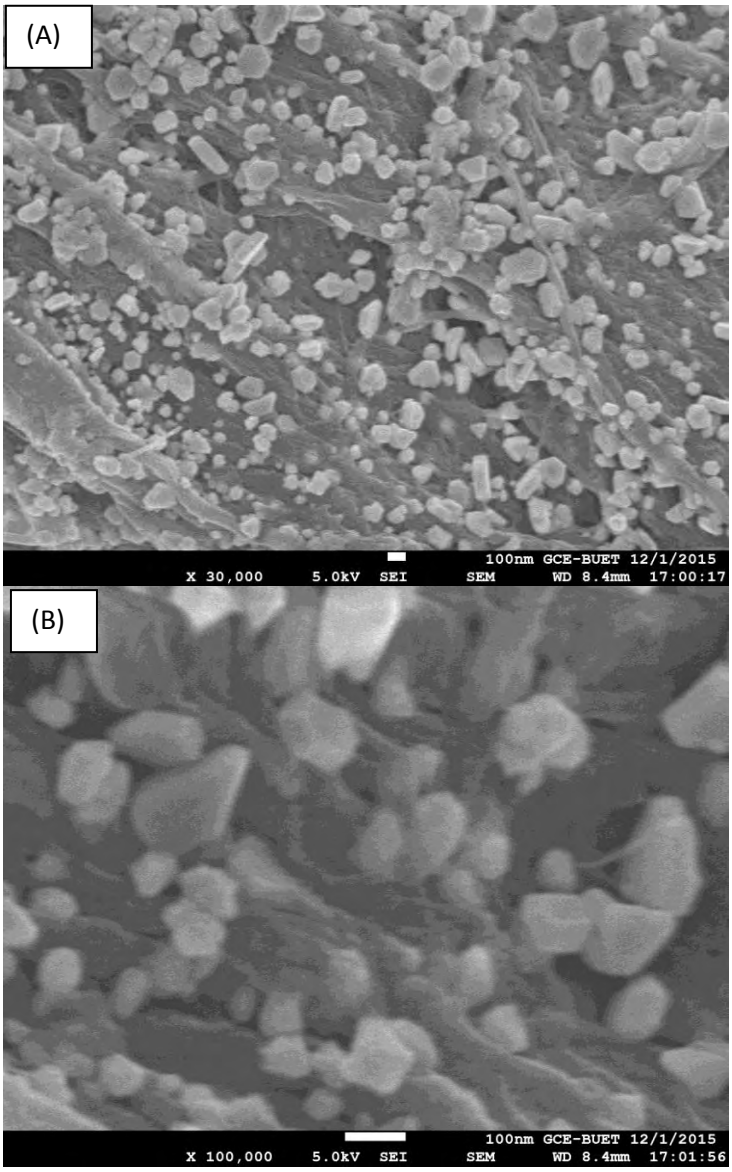

Fig. 3.13 SEM images of AgNP-paper of contact time 12 hours with Ag^{+} solution
(A) Resolution x30000 (B) Resolution x100000

At last, we conduct the 18 hours deposition study. Fig. 3.14 shows the 18 hours AgNPs deposited paper. From the Fig. 3.14 it is clearly seen that the deposition is dramatically decreased and the more clusters appear rather than smaller nanoparticles. In order to explain

this fact, we report that one of the possible reasons for this would be when the silver nanoparticles was produced

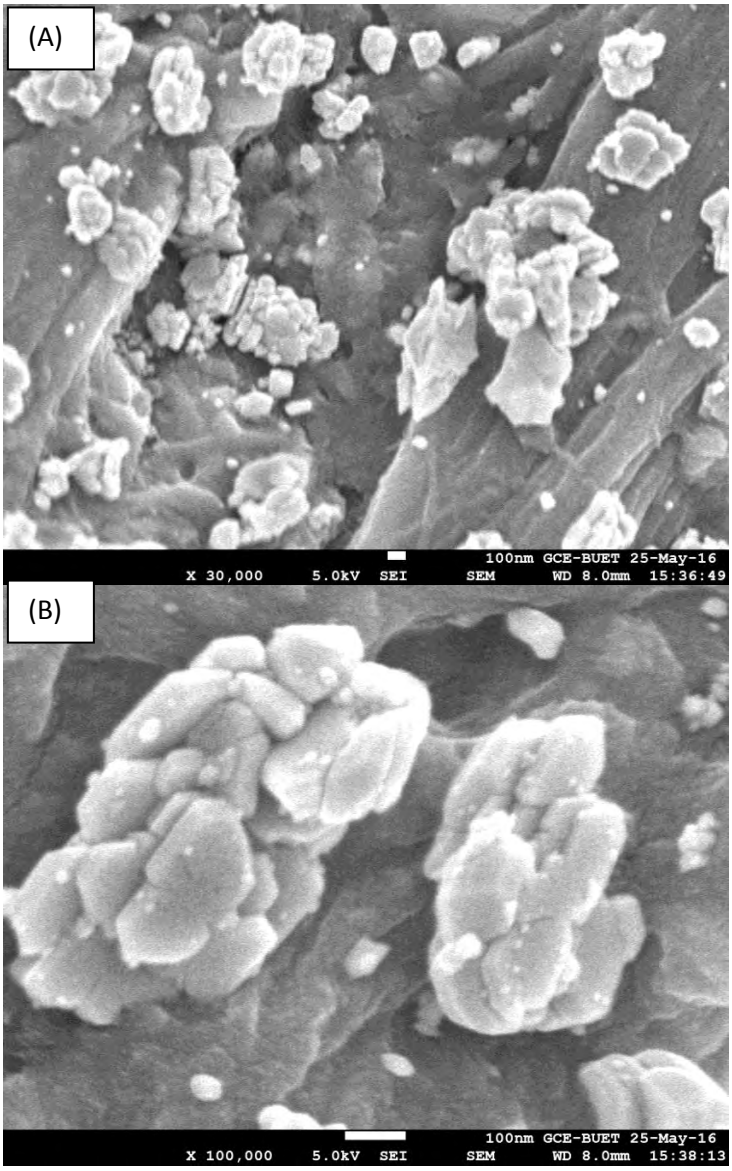

Fig. 3.14 SEM images of AgNP-paper of contact time 18 hours with Ag^{+} solution
(M) Resolution x30000 (N) Resolution x100000

by in situ reduction process by the catechol groups of dopamine molecules, it became bigger as the contact time of ammoniacal silver solution increase. So, after holding a particular sized

particle, it is given off into mother solution when it is getting bigger at that particular sized particle (Fig. 3.15).

Fig. 3.15 Optical image of the deposition of AgNPs onto dopamine added paper surface (left one) deposition time one minute (right one) deposition time 12 hours.

The above right side optical image clearly indicates the presence of AgNPs in solution after 12 hours. The possible explanation is that, due to the adsorption of dopamine molecules by cellulose threads and after reduction of Ag^{+} ions as AgNPs by catechol groups, the desorption of the adsorption dopamine molecules with AgNPs (see deposition study).

3.5 X-ray Photoelectron Spectroscopy (XPS) Study

In order to assess and confirm the bonds that was formed during the various treatment with cellulose glucose unit and the incorporated groups and the electronic state of the deposited particles, we use the most complicated, expensive, surface sensitive technique that is X-ray photoelectron spectroscopy.

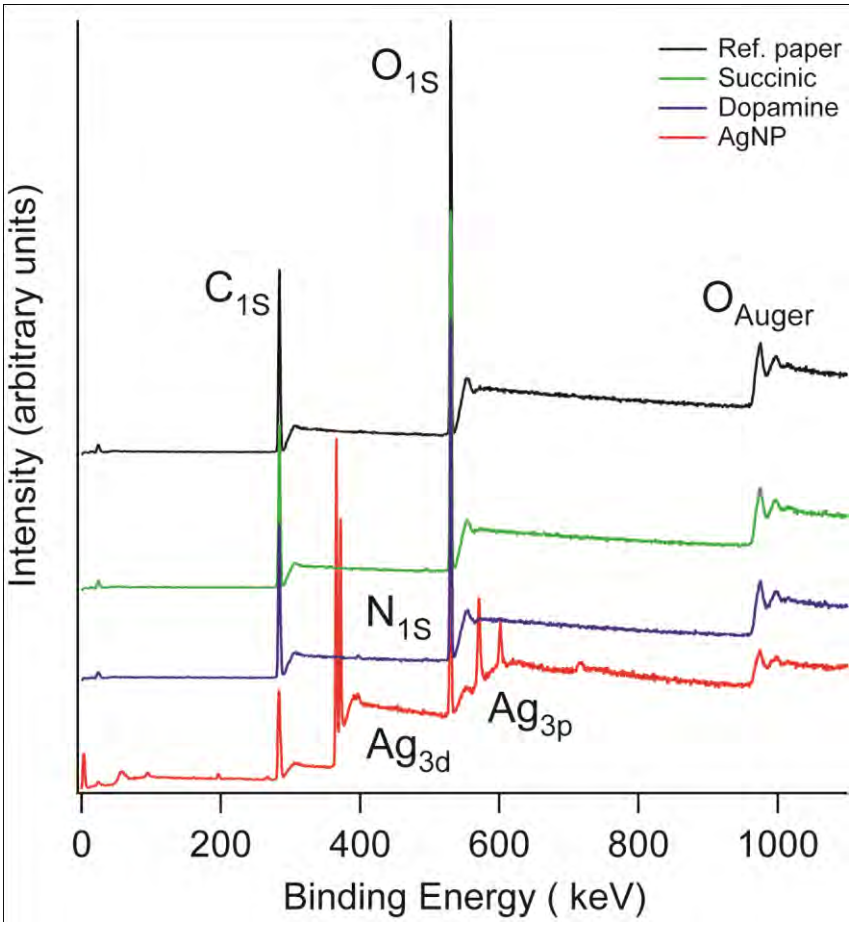

Fig. 3.16 Widescan XPS spectra of reference, succinylated, dopamine and silver added paper.

The widescan XPS spectra of reference, succinylated, dopamine and AgNPs deposited paper is shown in Fig. 3.16 From the Fig.ure the XPS spectra of reference and succinylated paper are similar because there is no elemental change happens during succinylation process. The reference paper is made of cellulose which is a polymer of glucose units. As H-atom is not detectable in this technique [4], the peak of carbon and oxygen are seen in reference and

succinylated paper spectra. If we move down to the dopamine added paper, here also the carbon and oxygen peaks are dominating but the nitrogen 1s peak also seen in the spectrum. The N 1s peak is very small [5] but significantly visible in high resolution spectrum. The last one is the AgNPs deposited paper where significant change has appeared due to the addition silver 3p 3d peaks. All the four spectra have a common oxygen auger electron spectrum.

3.5.1 High Resolution Carbon 1s Spectra

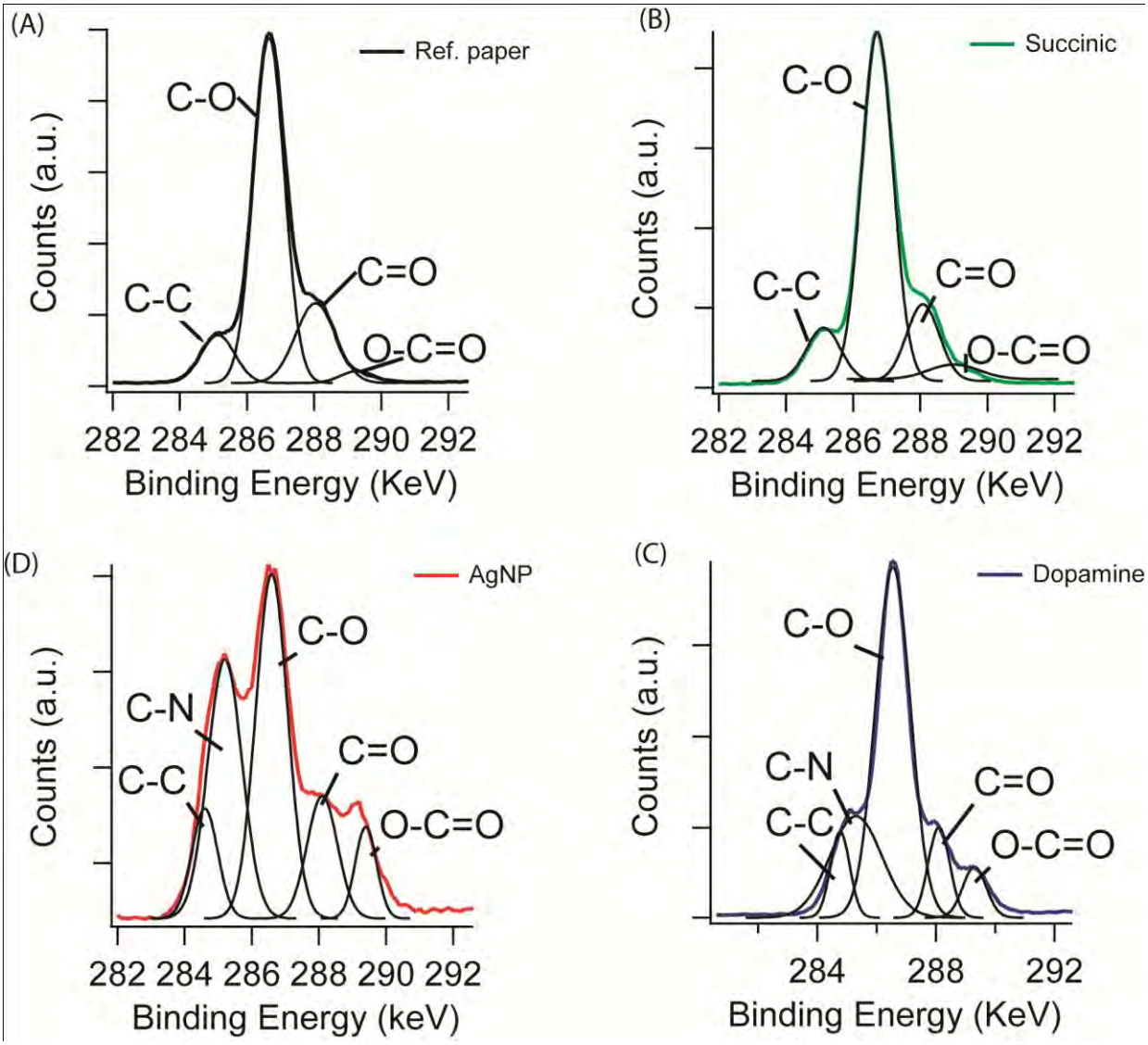

Fig. 3.17 High resolution carbon 1s XPS spectra (A) Reference paper (B) succinylated paper (C) Dopamine added paper (D) Silver nanoparticles (AgNPs) added paper.

The high resolution carbon 1s spectra of reference, succinylated, dopamine and AgNPs added paper are shown in Fig. 3.17. The carbon 1s peak of reference paper can be deconvoluted into four components peak. According to the binding energy these peaks are assigned to the carbon of C-C (285.1 KeV), C-O (286.6 KeV), C=O (288.0 KeV) and O-C=O (289.3 KeV) [6-8]. The component peak at 285.1 KeV is probably due to presence of hydrocarbon contamination. The unfunctionalized cellulose paper shows characterized peaks at 286.6 KeV and 288.0 KeV. As cellulose is a natural polymer, little oxidation occur readily at the surface which explains the carbonyl peak at 289.3 KeV [9]. After succinylation nothing changes at the peak position occur and remain the same as reference because no new types of bonds are formed. If we move down to dopamine added paper significant changes appear at the peak position and therefore now the peaks can be deconvoluted into five components which are assigned to C-C (284.7 KeV), C-N (285.3 KeV), C-O (286.5 KeV), C=O (288.1 KeV) and O-C=O (289.3 KeV) [10]. The new peak at 285.3 KeV and as we used low pH-5.5 condition where the dopamine cannot undergo polymerization which indicates that the coupling reaction between –COOH and $–NH_2$ we have done earlier in our experiment had successfully happened. After addition of AgNPs onto the paper surface by in situ reduction process no major change appear in the peak positions rather than intensity increase. This is probably due to the fact that now the surface is covered with a layer of AgNPs which block the dominating groups which previously suppress the surface functional groups.

3.5.2 High Resolution Nitrogen 1s Spectra

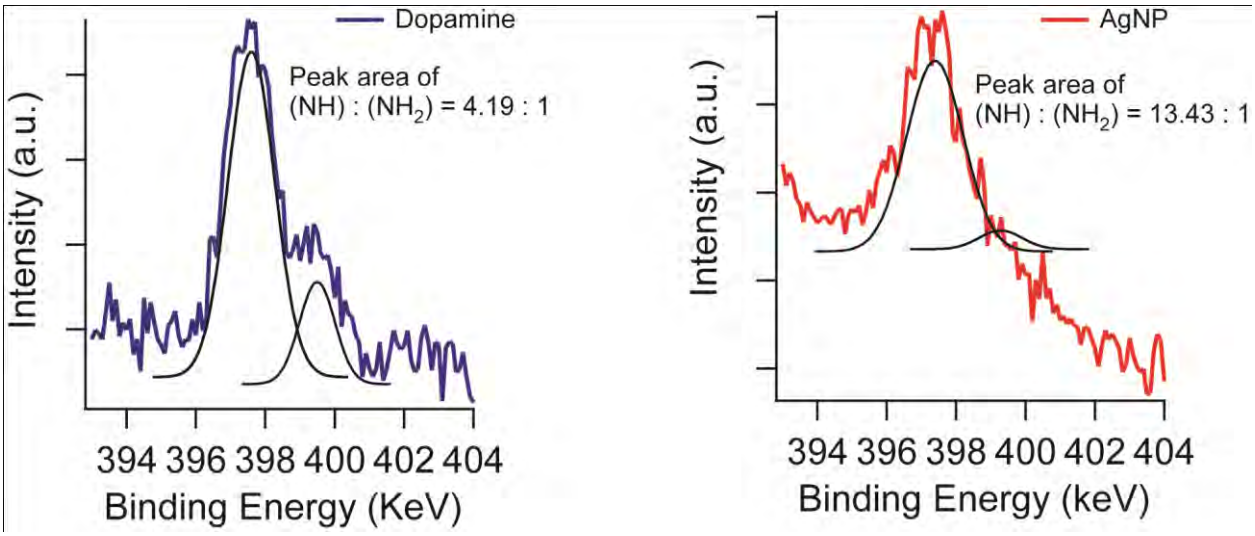

Fig. 3.18 High resolution N 1s XPS spectra of dopamine and AgNPs added paper.

The high resolution XPS spectra of nitrogen 1s peak of dopamine and silver nanoparticles added papers are shown in Fig. 3.18. Both the spectra have a common peak at 397.6 KeV which indicates that the binding energy peak of =NH groups [11, 12] of amide that is formed during coupling reaction between dopamine and succinic acid group. The peak at 399.28 KeV revealed the binding energy peak of $–NH_2$ group. This is probably due to very small amount of adsorption of dopamine molecules by the cellulose substrates. The event of adsorption of dopamine by the cellulose threads is also cleared by the N 1s spectrum of AgNP-paper. It is seen from the spectrum that the peak at 399.28 KeV which present in dopamine added paper is disappeared in AgNP-paper. This was happened because the catechol groups of dopamine reduced the Ag^+ ions bind them as silver nanoparticles and afterward when the size of the particles was big enough then the particles went into the ammoniacal silver solution as silver nanoparticles are highly dispersible in water. (see silver deposition study)

3.5.3 High Resolution Silver 3d Spectra

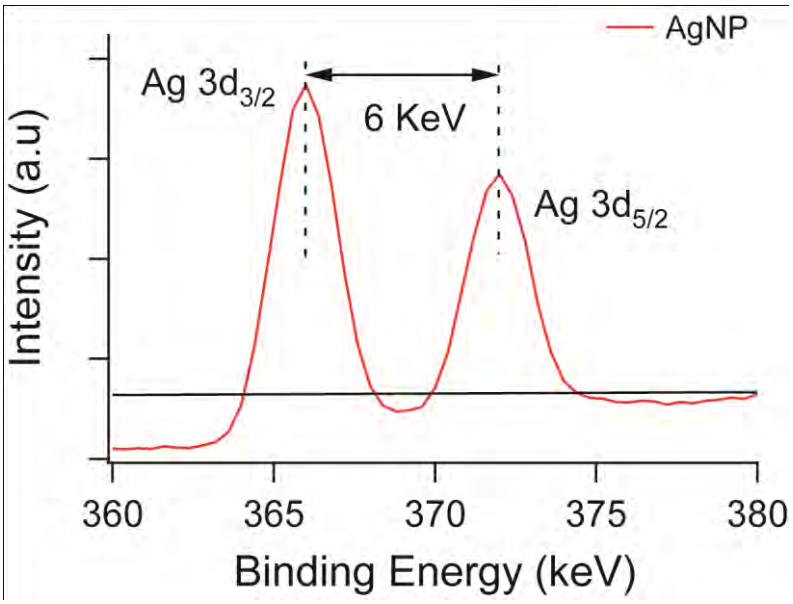

Fig. 3.19 Representative XPS spectra taken for an AgNP immobilized paper showing high resolution Ag 3d spectrum.

The oxidation state of immobilized silver nanoparticles (AgNPs) onto paper surface was analyzed using XPS. The high resolution Ag 3d XPS spectrum is shown in Fig. 3.19. The binding energies of Ag $3d_{3/2}$ and Ag $3d_{5/2}$ at 366 KeV and 372 KeV, respectively and the splitting of the 3d doublet of Ag was 6 KeV, indicating the existence of AgNPs at their Ag^0 state [13]. The peak at 366 KeV is also ascribed to the Ag atom bound to oxygen containing group [10, 14]. The high resolution Ag 3d spectrum could not be deconvoluted including three additional peaks at binding energies of 367.7, 368.8, and 374.2 eV, thereby indicating the abesence of small proportions of AgO, and Ag_2O along with metallic silver [15, 16].

3.6 Release of AgNPs from AgNP-paper

For silver release study we use 12 hours and 18 hours AgNP-paper. The total individual deposition of the two papers was 0.587 mg cm^{-2} and 1.02 mg cm^{-2} respectively. The release of silver nanoparticles in deionised water (DI) at ppm level after different time intervals is shown in table 3.2.

Table 3.2: The silver ions release from 4 cm^2 AgNP-paper in 200 ml deionised water.

Collection time of AgNPs containing solution	18 hours deposition AgNP-paper	12 hours deposition AgNP-paper
	Silver ion concentration (ppm)	
1 Hour	0.045 ± 0.004	<0.01
4 Hours	0.050 ± 0.003	0.023±0.002
8 Hours	0.100 ± 0.001	0.616±0.034
12 Hours	0.176 ± 0.001	1.205±0.060
18 Hours	0.491 ± 0.003	1.395±0.069

From the above table, it is seen that the concentration of silver ion release in DI water after 60 minutes in 18 h deposition AgNP-paper is 0.045 ppm or 45 ppb. If we consider 2 cm^2 area of our paper the concentration would be 22.5 ppb which is reasonably less than previously reported immobilized AgNP-glass [17]. This indicates that the AgNPs that is immobilized onto the surface of paper held so robustly that leaching of AgNPs did not happened at all. If we see the time intervals between 1h and 4 h the silver ion concentration increases is little and then goes gradually with increase of time. This is probably related with the morphology of the 18 hours AgNP-paper surface. We saw in the FE-SEM images of 18 h AgNP-paper where the smaller silver nanoparticles ware less than the big agglomerates. The smaller

AgNPs undergo readily dissolution than the bigger agglomerates [18]. This explains the initial increase of Ag^+ ion concentration into the solvent after one hour. The dissolution of AgNPs was continuing by the contribution of other big agglomerates with the increase of time. If we look at the 12 hours AgNPs deposition paper, after one hour the release of Ag^+ is very small and is less than 0.01 ppm. If we look at the FE-SEM image of x100000 resolution of 12 h AgNP-paper then we will

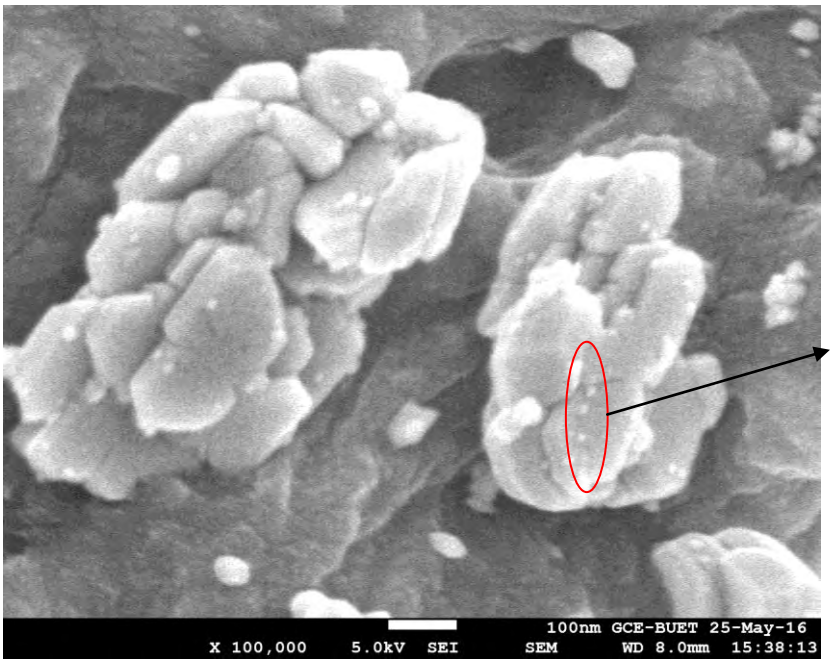

Fig. 3.20 SEM image of AgNP-paper of resolution x100000 and Ag^+ solution contact time 18 hours.

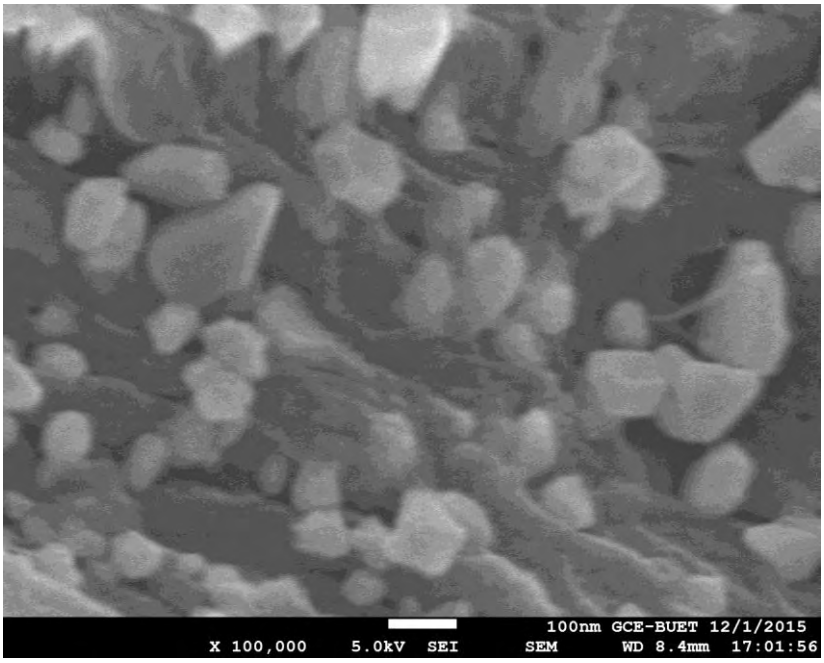

Fig. 3.21 SEM image of AgNP-paper of resolution x100000 and Ag^{+} solution contact time 12 hours

see that the size of the nanoparticles is almost similar within 100 nm range. On the other hand, in the 18 h AgNP-paper we see some small nanoparticles of size range 5-20 nm and this explain the readily dissolution and increased Ag^{+} ions concentration of 18 h AgNP-paper than 12 h paper. After 1 hour the 12 h AgNP-paper follow the trend of 18 h AgNP-paper.

3.7 Effect of time on silver nanoparticles (AgNPs) deposition

This study was carried out to confirm the observation that was found in FE-SEM study. We saw in FE-SEM study that the deposition of 5 minutes is quite low, 15 minutes moderate, 4 and 8 hours show some similarity, 12 hours had great loading and finally 18 hours again decreased due to the formation of big clusters. Herein, we prepared five samples of AgNP-paper by changing the contact time between dopamine added paper and ammoniacal silver solution (same as before) that is 15min., 4h, 8h, 12h, 18h and three samples without modification whose contact time with ammoniacal silver solution were 15min., 12h, 18h for blank measurements. Table 4.3 shows the weight of AgNP-paper before and after burn.

Table 3.3: Weight of AgNP-paper of different contact time with Ag^+ solution before and after burning

Contact time with Ag^+ solution	Weight of AgNPs of the AgNP-paper before burning g	Weight of AgNPs of the AgNP-paper after burning g
15 min.	0.1249	0.0070
4 h	0.1268	0.0086
8 h	0.1268	0.0083
12 h	0.1287	0.0097
18 h	0.1258	0.0086

Table 3.4: Weight of blank-paper (unmodified) of different contact time with Ag^+ solution before and after burning.

Contact time with Ag^+ solution	Weight of AgNP-paper before burning g	Weight of AgNP-paper after burning g
15 min.	0.1104	0.0004
12 h	0.1091	0.0002
18 h	0.1107	0.0001

Clearly the table 3.3 indicates the different amount of deposition of AgNPs onto dopamine added paper surface when the contact time with ammoniacal silver solution varies. Thus, the observation of FE-SEM images again proves in this section. From the table 3.4, it can be

easily anticipate that our strategy and method we used was stunning than the previously reported methods [19-21] to immobilize silver nanoparticles. We also plotted the weight of AgNPs of the AgNP-paper with time before and after burning and got the following graphs. The two graphs have similar pattern. The probable explanation of the graphs is that, after the reduction of Ag^{+} ions by the catechol groups of dopamine the nucleation starts and this facilitates the rapid deposition of silver nanoparticles onto dopamine added paper surface. Therefore, the deposition is uniform and the size of the particles is reasonably small as seen in FE-SEM images. Since, the concentration is fixed in our experiment, after the nucleation as more and more Ag^{+} ions were reduced therefore the size of the particles increased with time [22]. This explains the initial increase of deposition after 4 hours and is also seen from the graph. It was also seen from FE-SEM image of 4 hours AgNP-paper that some multilayer aggregates appear on the paper surface. The aggregates were gradually undergone into the ammoniacal silver solution which was attached by dopamine molecules that are not undergone coupling with succinic groups. Adsorbed dopamine molecules when they cross the holding force range limit of adsorption with cellulose threads, undergo into ammonical silver solution with AgNPs. The colour change of the ammoniacal silver solution also indicates the release of nano-sized silver particles into the solution. The optical images are also shown in Fig. 3.24. This decreased the deposition of AgNPs onto the paper surface slightly after 8 hours. Afterwards, there is probably a sort of equilibrium was established between the released nano-sized particles and the immobilized particles onto the paper surface within 8 to 12 hours and this again increased the deposition. The decrease of deposition after 12 hours is probably due to the big agglomerates formation as seen in FE-SEM image and which again when cross the holding force range limit of adsorption undergone to the solution.

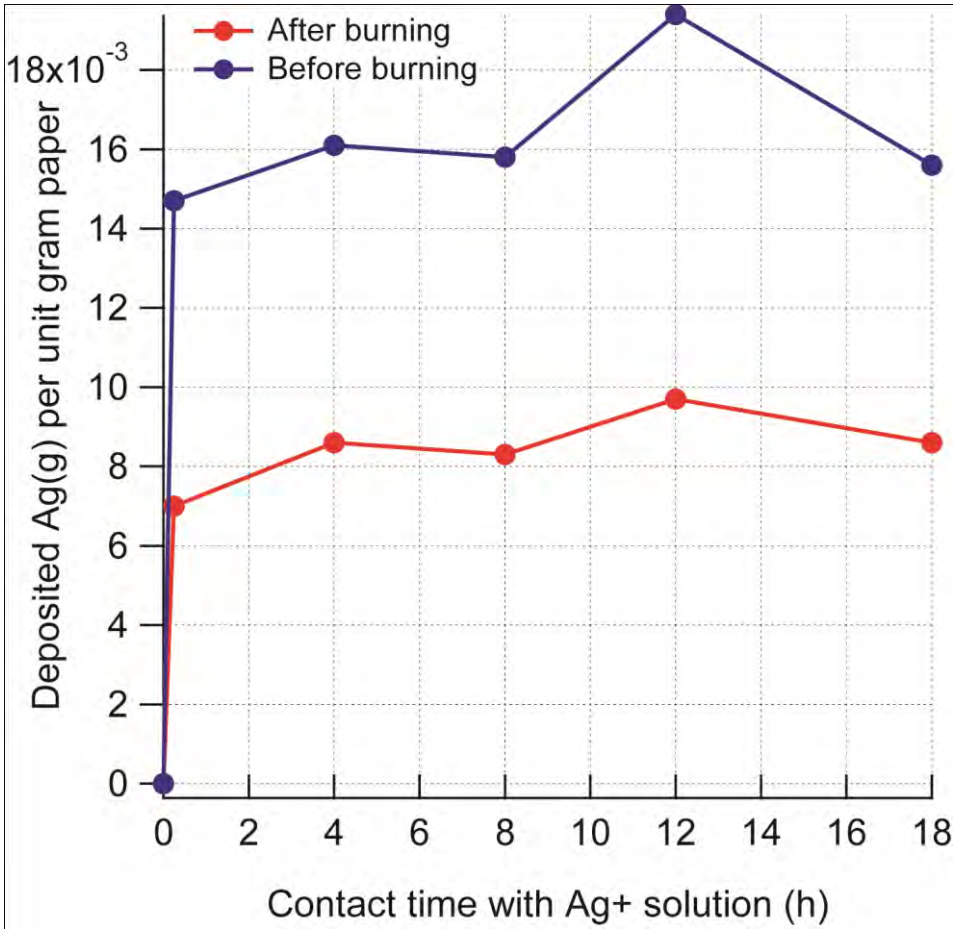

Fig. 3.22 Plots of the weight of AgNPs with time which was deposited onto AgNP-paper surface before burning and after burning.

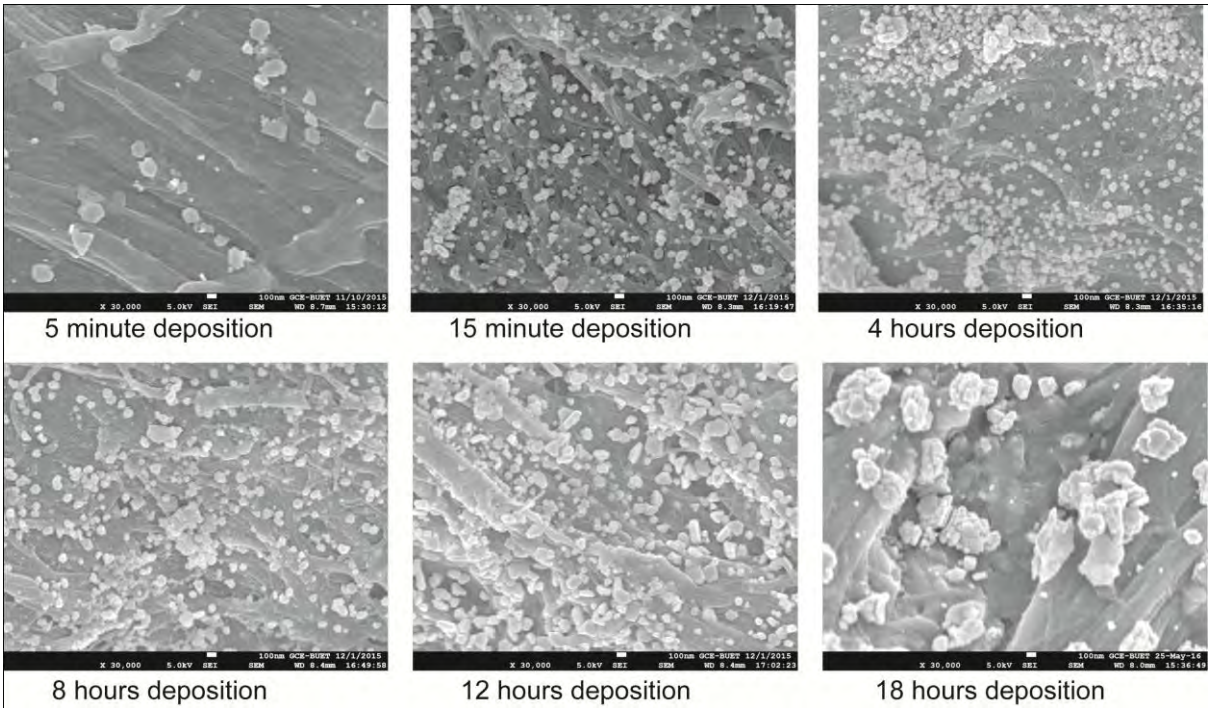

Fig. 3.23 SEM images of AgNP-paper of different contact time with ammoniacal silver solution.

Fig. 3.24 Optical image of ammoniacal silver solutions after reacting with dopamine added paper with different time intervals.

We also cross checked the solutions that are left after AgNPs addition to ensure the presence of silver nanoparticles with dopamine molecules in the solutions of different time intervals and the ammoniacal silver solution. We found the peaks at 425-430 nm range due to the surface Plasmon resonance of the nanoparticles [22] (Fig. 3.25). This clearly indicates that

the nanoparticles that were formed onto the surface due to in situ reduction undergo into the solution because of the dopamine molecules that cross the holding force limit of adsorption with cellulose threads. As expected the peaks at 295 nm indicate the presence of dopamine molecules in the solutions. The shifting of peak from 425 nm to 430 nm of AgNPs in solution indicates the irregularity of shape of AgNPs [23] that is started forming after 8h and is also seen from FE-SEM images.

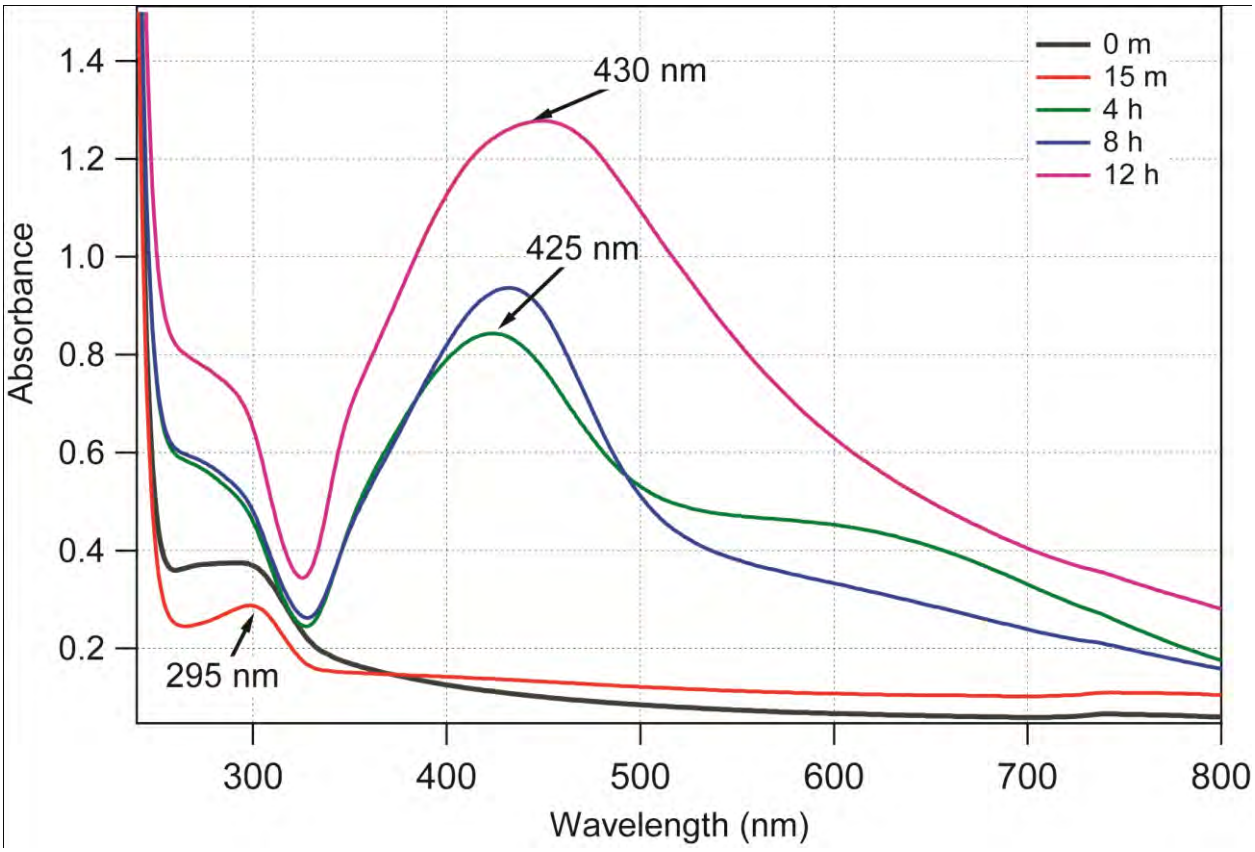

Fig. 3.25 UV-spectra of ammoniacal silver solutions after reacting with dopamine added paper at different time intervels.

3.8 Antimicrobial activity

3.8.1 Against bacteria

Antibiotic resistance is becoming a burning issue around the world. The number of bacteria resistant to antibiotics has increased in the last decade. Antibiotic-resistant bacteria can quickly spread, threatening the community with a new strain of infectious disease that is more difficult to cure and more expensive to treat. Antibiotic-resistant infections that are difficult to treat are becoming increasingly common and are causing a global health crisis and food security. However, recent study has shown that the silver nanoparticles can act against a very broad spectrum of bacterial and fungal species including antibiotic resistance stains preferentially [24]. So more and more silver nanoparticles based antibacterial materials have developed to fight against all these strains. Other alarming infections that have reported such as hospital associated infections known as nosocomial infections which is a increasing threaten of admitted patients. The increasing number of death around the world from nosocomial infections are also reported in the last decade. Report also said that, one of the possible solutions to prevent nosocomial infections is the practice of the use of hand hygiene [25]. These will motivated the researchers to develop a new antibacterial paper that will be available and used for hand hygiene and also can Fig.ht against antibacterial resistance strains. Like other developing country Bangladesh is also being risked with these increasing threatening. So, in this research we also try to develop an antibacterial paper which would be useful for preventing microorganism attack and thus ensure food security and hospitals related infections. In this context we are trying to Fig.ure out that does our synthesized silver nanoparticles based paper (AgNP-paper) either show antibacterial activity or not. We had tested our paper against some virulent bacteria which are antibiotic resistant. They cause diseases in fish and shrimp which are third most important source of foreign currency from agricultural products in Bangladesh. We use simple disk diffusion method to show the qualitative antibacterial test of our paper. Four different types of AgNP-paper are used in this purposes and all the AgNP-paper show excellent antimicrobial activity against the pathogens. The table 4.5 shows the zone of inhibition that is caused by the sterilized AgNP-paper disk after 24 h at 28°C and the optical images are shown in Fig. 3.26

Table 3.5: Values of zone of inhibition of antimicrobial activities of different AgNP-papers against some fish and shrimp pathogenic bacteria. (N.T.> Not Test)

Bacterial strain		Ratios between the diameter of zone of inhibition and AgNP-paper in mm scale			
		15 min.	8 h	12 h	18 h
Gram negative	*Vibrio pharahaemolyticus 2A1* (shrimp)	1.83	2.10	2.25	1.76
Gram negative	*Vibrio pharahaemolyticus 2A2* (shrimp)	1.83	2.43	2.30	2.28
Gram positive	*Enterococcus faecalis FF11* (fish)	-	1.93	1.58	2.24
Gram positive	*Enterococcus faecalis F1B1* (fish)	2.0	1.98	1.66	2.94
Gram negative	*Serratia marcescens 4V3* (shrimp)	-	N.T.	1.66	N.T.
Gram negative	*Proteus mirabilis NS34* (shrimp)	-	N.T.	-	N.T.

The above mentioned bacteria like *Vibrio pharahaemolyticus* species are responsible for sea food-poisoning, wound infections and cause disease to shrimp. So, controlling of *v. pharahaemolyticus* would be possible through using our AgNP-paper. The *Enterococcus faecalis* and *Serratia marcescens* species are commonly associated with wound infections, urinary tract infections, endocarditis and hospital associated infections (HAI) etc. The *s. marcescens* also cause catheter associated infections, eye infections, pneumonia and meningitis and it is reported that 1.4% HAI associated death in US is caused by this species. It is also alarming that, the *Serratia marcescens* is resistant to ampicillin, macrolides and first generation cephalosporins. But our AgNP-paper show excellent activity against these pathogens. So, it can be concluded that our AgNP-paper would be useful for various setting in medical applications such as antibacterial hand wipe to prevent nosocomial infections.

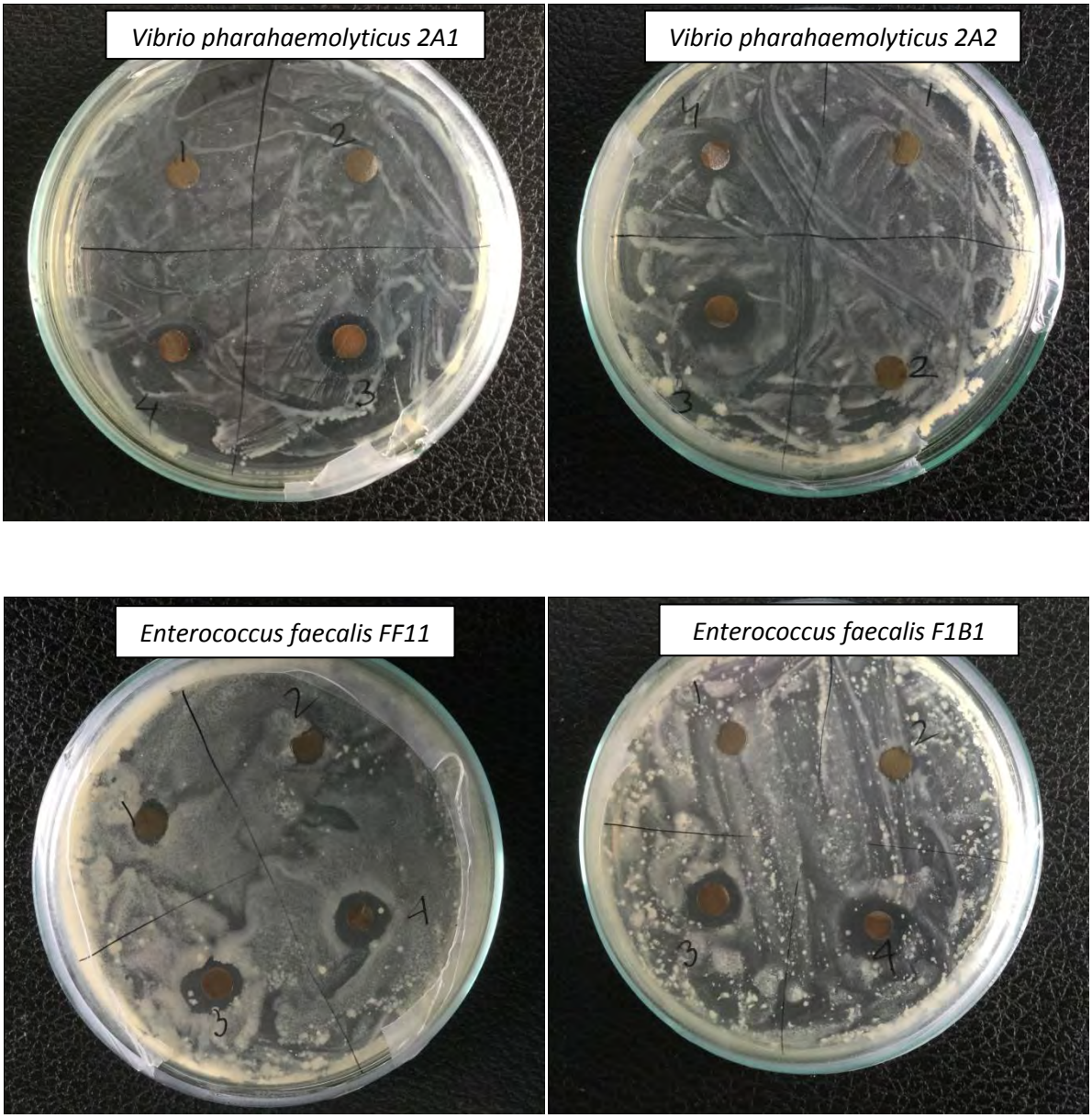

Fig. 3.26 Optical images of antibacterial activities of AgNP-papers against some antibiotic resistant bacteria.

3.8.2 Against fungus

Bangladesh is predominantly an agricultural country, depending mainly on crop plants, agricultural and forest products for its economic development. Because of its fertile land, crops play a vital role in economy of the country and agro-ecological conditions are favorable for the production of various crops. But still the yield of crops is often poor. This is because plant disease caused by different micro-organisms effect significantly in crop production.

„Boiled Rice" is our main meal. We eat „Boiled Rice" at least two times. Therefore, farmers all around this country grow paddy as their main cash crops. But, wheat cultivation has introduced among the farmers since 1940s. Now it is the second cereal crops in this country. Wheat is susceptible to several diseases. That is why it has got popularity since 1990s. But recent threaten of „wheat blast" disease has outbreak through some southern part of the country and is caused by a virulent fungal species named *Magnaporthe oryzae*. The disease is generally spread by infected seeds and airborne spores, and the fungus can survive in infected crop residues and seeds [26]. As a new antibacterial or would be new antifungal material to prevent the outbreak through the whole country, we plan to test our AgNP-paper against the fungal species to control them.

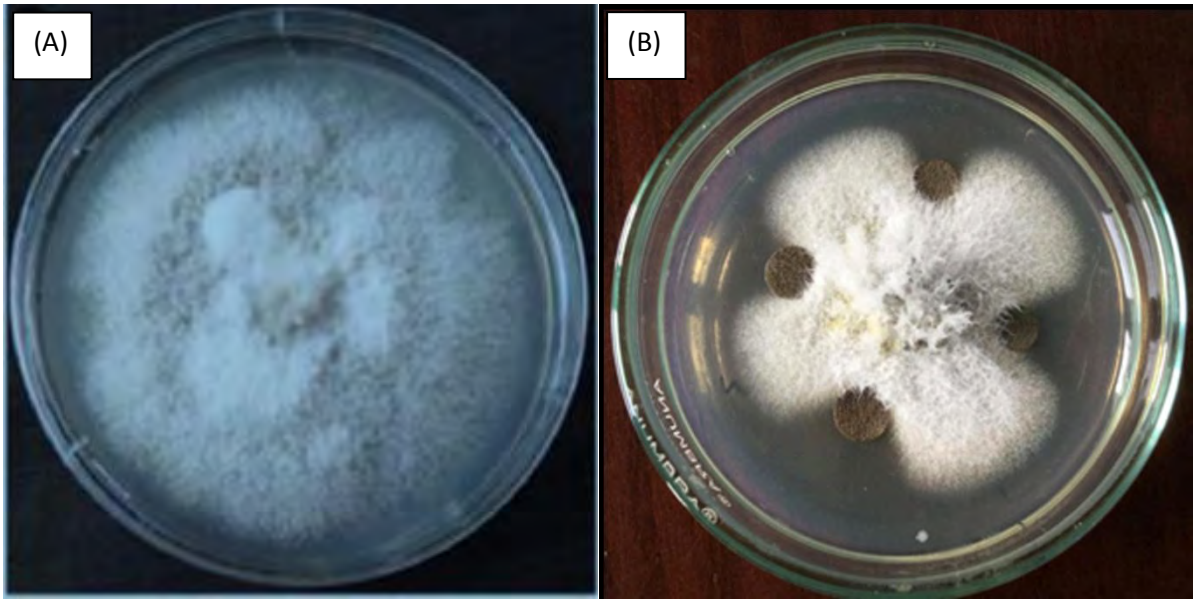

Fig. 3.27 Optical images of antifungal test of AgNP-paper against *Magnaporthe oryzae* (A) Culture of *Magnaporthe oryzae* on potato dextrose agar plate after 14–21 days of inoculation. (B) Antifungal activity of AgNP-paper after seven days.

Surprisingly, our AgNP-paper shows excellent antifungal activity against the species after incubating seven days in dark at 28^{o}C (Fig. 3.27). So, we can hope that our synthesized AgNP-paper would be useful to preserve or protect the wheat crop from this virulent fungal species.

3.9 Toxicity test

Toxicity tests are conducted to evaluate the nature and degree of harmful effects of a chemical substance. The studies are designed to obtain as much information as possible so that the toxic effects are predicted. The nature of each test is determined by the intended use of the chemical to be studied. As Bangladesh is an agricultural country, variety of agricultural crops as cereal, fruits, vegetables are grown all over the year in this country. One of the major problems of these crops is that some of them are rotting. So starting from farmer, stockiest and finally the seller use numerous types of chemicals such as formalin, DDT etc. which are toxic to humans, plants and environment. In our research, we choose cellulose paper which is known to be most available, biocompatible and nontoxic to any species and immobilized silver nanoparticles which are also known nontoxic to human health onto it. Herein, the novel method we have used and the whole procedure couldn‟t give any chance to make our final AgNP-paper to be toxic. However, resting all this things we try to find out the possible toxicity of our AgNP-paper by applying directly the AgNP-paper into seed germination and plant leaf.

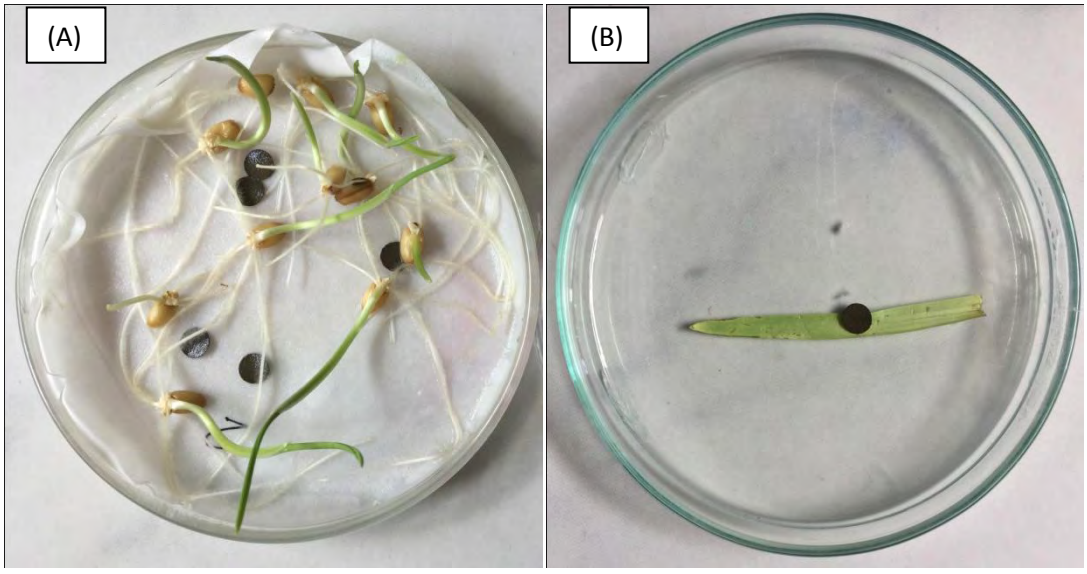

Fig. 3.28 Toxicity test experiment (A) Wheat seed germination with AgNP-paper (B) Barli leaf with AgNP-paper.

From Fig. 3.28(A) it is clearly seen that, the wheat seed germination didn‟t hamper in the presence of AgNP-paper and therefore we hope that our AgNP-paper can be used as preserving or wrapping materials for preserving this types of cereal crops or other fruits and vegetables without causing any toxicity of this food materials. Fig. 3.28(B) reveals that AgNP-paper has no toxic effect on plant cells or tissues and if it had then the barli leaf would had black spot around the AgNP-paper disc. Thus our AgNP-paper may be exploited in preserving rotting fruits and vegetables.

3.10 Conclusion

In this research we successfully synthesized antibacterial paper through novel mussel inspired strategy immobilizing silver nanoparticles onto cellulose paper matrix. The morphology of the nanosilver immobilized paper was characterized by atomic force microscopy (AFM) and scanning electron microscopy (SEM). We also found from SEM study that, the deposition and size of silver nanoparticles onto paper matrix is depended on the contact time between the ammoniacal silver solution and dopamine added paper. We also try to Fig.ure out the optimum conditions to get a better quality paper. The elemental composition and oxidation state of nano-sized silver was characterized by x-ray photoelectron spectrosocopy (XPS). The results show that the successful addition of succinic group and after coupling the amide bond between succinic group and dopamine molecule and the bonding between nanosilver and catechol group of dopamine. The XPS results also reveal that the oxidation state of deposited nanosilver is zero valence that is deposited nanosilver is in metallic state. After the characterization we tested the antibacterial paper against some virulent antibiotic resistant bacteria and fungal species. The antibacterial and antifungal tests reveal the excellent antibacterial and antifungal activity of our synthesized nanosilver coated paper. Therefore, we hope that our antibacterial paper would useful for making antibacterial product such as antibacterial hand wipe which is seriously needed in hospitals and clinic to prevent hospital associated infections. The antifungal activity of our paper can be exploited to prevent the loss that is caused by various microorganisms in our agricultural crops and fruits. Passing in toxicity test of our antibacterial paper make it more acceptable to use it in numerous applications.

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