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By Sonal S. Padalkar

Entitled Proteins and Natural Biopolymers as Templates for Inorganic Nanomaterial Synthesis

For the degree of Doctor of Philosophy

Is approved by the final examining committee:
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Prof. Tim Sands
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PROTEINS AND NATURAL BIOPOLYMERS AS TEMPLATES FOR INORGANIC NANOMATERIAL SYNTHESIS

A Dissertation
Submitted to the Faculty
of
Purdue University
by
Sonal S. Padalkar

In Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

May 2010
Purdue University
West Lafayette, Indiana
UMI Number: 3413833

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I would like to acknowledge the financial support provided to me through the start fund given to Dr. Stanciu. I would like to thank Dr. Stanciu for serving as my major advisor. I would like to thank Professors Tim Sands, Eric Stach and Edwin R. Garcia who served on the advisory committee. I acknowledge the support and help given by Dr. Rochet. A sincere thanks to Seung Min Kim and John Hullenman for helping me during the entire course of my work at Purdue. A special thanks to all my labmates, Robert Colby, Ravi Sinha and Yu-Ho Won for their help. I would also like to thank the undergraduate students, Kara, Thejaswi, Praveen and Kimberly who worked with me.

On a personal note, I would like to thank my parents and family for their support. Finally, I would like to thank my husband Parijat Deb without whom this PhD would have not been possible.
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ABSTRACT

Padalkar, Sonal S., Ph.D., Purdue University, May, 2010. Proteins and Natural Biopolymers as Templates for Inorganic Nanomaterial Synthesis. Major Professor: Dr. Lia Stanciu, School of Materials Engineering.

The synthesis of one dimensional (1D) structures, using the bottom up technique has gained much attention in the past few years. This is due to the unique advantages of the synthesis method. The bottom up synthesis route for the fabrication of 1D structures utilizes mild experimental conditions, short experimental time, relatively inexpensive precursors and does not require a precise control of process variables. Moreover, the biotemplate can be functionalized which helps in the proper positioning of the 1D structures in complex circuits. In the present work, alpha synuclein protein was used as a model template for the fabrication of metallic (silver, platinum) and semiconducting (cadmium sulfide, lead sulfide, zinc sulfide) nanowires. The lateral dimensions of the nanowires could be controlled by varying the process variables. Further, this work was extended on to a cellulose template. The cellulose template is an inexpensive template, compared to proteins, and is abundantly available in various forms. Later, the biotemplated Silica and Titania nanowires were utilized for a biosensing application. The synthesized 1D structures show promise in various fields ranging from electronics, catalysis to biosensing.
CHAPTER 1. INTRODUCTION

The properties of nanoparticles and nanowires offer the potential of enhanced electrical, mechanical and thermal properties when compared to their bulk counterparts [1-9]. As the nanowire diameter gets close to or smaller than the scattering lengths of electrons, photons and phonons, the properties of the nanomaterial significantly differ from those of the bulk materials. At critical dimensions below 10 nm, quantum size effects become dominant, holding promise for breakthrough in nanoelectronics. The nanocrystal and nanowire dimensions in the range of 1-25 nm match well the size scale of biological molecules and offer opportunity in understanding the bottom-up nanofabrication nature employs. Recently [10-14] much progress was made in the area of nanomaterials processing using the bottom-up approach, by self-assembly of systems with molecular recognition capacities. Biological systems possess a high degree of organization from molecular building blocks (peptides, aminoacids, proteins, nucleic acids) and are perfect models for bottom-up strategies for controlled material synthesis. Their molecular recognition capabilities, combined with the specificity towards certain ions and molecules, can be used to precisely control the fundamental processes involved in materials synthesis and processing, such as: phase stability, nucleation and growth, pattern formation and assembly [12-17]. The thermodynamic control over formation processes of self-assembled materials is another major advantage that often makes
high yields of the synthesis product possible. For decades, by molecular selection, nature fabricated countless building motifs. This information and the motifs, nature offers can now be used to build a basic engineering and scientific knowledge foundation to be used in nanoscience and nanotechnology.

The high surface-to-volume ratio at the nanoscale requires a thorough understanding of charge localization, surface diffusion, chemical reactions and electron scattering at atomic scale. As the scale length decreases, these problems become more critical to designing functional materials for electronic and medical devices. Our understanding of the self-assembly mechanisms that control the exact positions of the individual molecular building blocks in biological scaffolds, and their interconnection is still not complete. In trying to mimic the structure and binding properties of biomolecules, one approach is to design and synthesize organic molecules. However, synthesis of such large and structurally complex organic molecules in which covalent bonding prevails, is extremely difficult and time consuming. Therefore, understanding the self-assembly in systems inspired from nature, where interactions are predominant weak and noncovalent is necessary for the modeling and practical use of biological molecules in nanotechnology. In addition to the challenges imposed by operating at nanometer scale lengths, interfacing biological with inorganic systems imposes challenges that require new bottom-up approaches that have to be designed and optimized with respect to size and shape control and structural order.

At the molecular level, handling and controlling the components of the system being studied becomes a challenge. Therefore, the necessity of an interdisciplinary
approach that combines chemical synthesis, characterization, biochemical techniques and computational analysis becomes apparent.

The goal of this thesis research was to contribute significantly to the advancement of research, knowledge, and learning the biomolecule assisted nanomaterials processing by combining active molecules, nanotechnology and chemical design.

To achieve the goal of the project, the research addressed the following objectives:

1. To develop a structure-properties correlation in systems involving peptide assisted nucleation and growth of metal nanowires and nanoparticle chains. Identification of the properties of nanowires and nanoparticles at the nanoscale can provide the basis for future design of nanowire arrays with improved properties than their bulk counterparts.

2. To develop strategies for the discovery of natural templates alternative to proteins, DNA and viruses that are cheaper and widely available and that could be used as scaffolds in biomineralization and synthesis of metallic and semiconducting nanostructures.

3. To use the synthesized nanostructures for a biosensing application. The synthesized nanostructures provide a high surface area, relatively inexpensive and a biocompatible platform for the immobilization of enzymes used in a biosensing application.

Chemical and structural complementarities of molecular building blocks are the most important concepts when studying self-assembly processes. The dominant interactions between residues are weak, physical bonds, such as hydrogen bonds. In aqueous solution, peptides have the ability to form beta sheet structures (Figure 1.1). The beta sheet was discovered by Pauling and Corey in 1951 [18] and is part of the secondary structure of proteins. Its structure is composed of several amino acid sequences within the
same polypeptide, which are parallel, adjacent, and oriented in such a way that hydrogen bonds can form between the strands. The hydrogen bonds form between an N-H group in one chain and an adjacent C=O group in the other, parallel, strand. Since the amino acid sequence extends along the whole structure, there is a cumulative effect of several such hydrogen bonds, which give the beta sheet structural stability. The cumulative effect of multiple hydrogen bonds arranged in this way contributes to the sheet's stability, structural rigidity and integrity. Depending on how the amino acid residues in the side chains are arranged in the beta sheet, it is possible to arrange its structure in such a way that one of its sides is hydrophobic, while the other side is hydrophilic and polar [19-23]. When in an aqueous environment, the hydrophobic side tries to go away from the water, while the hydrophilic side orients towards the water, on the sheet’s surface, allowing for self-assembled structures through their complementary ionic surfaces to remain stable.
The self-assembly process can be precisely designed by controlling the charge arrangements of the amino acid residues on the beta sheet surface. Peptide molecules containing both polar and nonpolar groups can be induced to assemble into various desired geometries, such as micelle aggregates, vesicles, bilayers or nanofibers [24-27]. Based on their beta sheet motifs, proteins are folded into unique structures when they are in their normal functional state. When certain polypeptides meet the required conditions for self-assembly into fibrils, 1D protein structures are formed. The polypeptide based nanowires are mainly composed of adjacent beta sheets bonded into a twisted fibrillar structure by hydrogen bonding. These 1D structures are generically called amyloid fibers and can be formed both in-vivo, by misfolding of pathogenic proteins, as well as outside the body, by self-assembly of some nonpathogenic proteins. The capacity of some polypeptides to self-assemble into fibrillar structures is called amyloidogenicity. The
presence of amyloid fiber assemblies in different parts of the human body can lead to pathologies such as Alzheimer’s disease [28-30]. Recently, it was shown that several non-pathologic polypeptides can self-assemble into amyloid-like fibrilar structures [31-35]. One dimensional nanostructures are suggested to have a wide range of applications in nanotechnology serving as nanofibers or nanoscaffolds [36, 37]. Although amyloid-like peptide nanofibers can be formed starting from various groups of proteins, unrelated from a structural point of view, it has been shown in literature that all the resulted amyloid fibrils share similar structural properties [38-41]. Although formation of amyloid fibrils is a generic property of the polypeptides, only peptides with a specific amino acid sequence can self-assemble into similar fibrils. Furthermore, certain proteins can self-assemble into amyloid-like 1D structures in conditions that are different from those necessary for other proteins to form amyloid fibers. Fibril formation is believed to be a nucleation and growth process, where the proteins change their structure to form nuclei that can subsequently grow into nanofibers. However, there are still uncertainties in understanding the exact mechanism involved into the self-assembly of various proteins into amyloid fibrillar materials and no comprehensive and coherent study of the mechanisms of self-assembly of fibrillar biological 1D materials is available to date[42-48].

The alpha synuclein protein is of interest due to its capacity to self-assemble into long amyloid-like fibrils under certain processing conditions, such as in the presence of transition metal ions. The structure, properties and synthesis of alpha synuclein protein is described in the following chapter.
CHAPTER 2. ALPHA SYNUCLEIN PROTEIN TEMPLATE

2.1 Structure and Characteristics of the Alpha Synuclein Protein Template

The alpha synuclein protein has a capacity to self-assemble into long fibrils under certain incubation conditions. Alpha synuclein is a 14.5 kDa protein found in the human brain [49]. This protein has been shown to self-assemble into fibrillar structures in the presence of certain transition metals [19, 51]. When fibrilization finds favorable conditions in-vivo, it leads to diseases such as Parkinson [49]. Under controlled incubation conditions in-vitro, alpha synuclein has the capacity to self-assemble into fibrils through the formation of extended beta sheets [52-54]. These fibrils are polypeptide based fibrils and are mainly composed of adjacent beta sheets bonded into a twisted fibrillar structure by hydrogen bonding. The structures are generically called amyloid fibrils and the capacity of the polypeptides to self-assemble into fibrillar structures is called amyloidogenicity. Although the mechanism is not completely elucidated, the fibril formation is believed to be a nucleation and growth process, where the proteins change their structure to form nuclei that can subsequently grow into nanofibrils. The self-assembly process can be precisely designed by controlling the charge arrangements of the amino acid residues on the beta sheet surface. Although the basic concept of self assembly is quite clear, there are still uncertainties in understanding the exact mechanism involved [52-54]. The diameter of these fibrils is ~8 nm and the length ranges between 500 nm to 1μm. This capacity of alpha synuclein
to form polypeptide fibrils with dimension as mentioned can be used as construction tools for the assembly of nanowires. The synuclein family of proteins are highly ordered structures and have a high net negative charge, strong hydrophobicity and specificity towards some ions and molecules [49-51] which further makes the alpha synuclein protein fibril an ideal template for the fabrication of 1D structures. Figure 2.1 shows a transmission electron microscopy (TEM) image of an alpha synuclein fiber. The fiber appears uniform and smooth along the length and has a twisted morphology.

![Figure 2.1. TEM image of an alpha synuclein fibril](image)

2.2 Synthesis of Alpha Synuclein Protein Template

In the formation of alpha synuclein fibrils, the native alpha synuclein monomers when dissolved in the phosphate buffer transform into soluble oligomers. These oligomers may then form protofibrils, which finally transform to alpha synuclein fibrils.
The exact relationship between the formation of smaller oligomers, larger protofibrils and the completely formed alpha synuclein fibrils is unclear [55].

For the self assembly of alpha synuclein fibrils, purified E46K alpha synuclein was chosen due to its capacity to self assemble at a faster rate. The alpha synuclein protein was dissolved in a fibrillization buffer (phosphate buffer having a pH~7.4 and containing 0.02% (w/v) NaN₃). The protein solution was filtered through a 0.22 μm nylon spin filter followed by a Microcon -100 spin filter which produced a stock solution depleted of aggregates. The protein solution was dialyzed against its own buffer to equilibrate its pH. This protein solution was later diluted in a fibrillization buffer to a final concentration of 100 – 300 μM and incubated at 37°C for 12 – 96 hr with gentle rolling in a tissue culture drum. Figure 2.2 shows a schematic representation of the synthesis process.

![Figure 2.2. Schematic representation of the synthesis process for the formation of the alpha synuclein protein template.](image)

Figure 2.3 shows TEM images of the alpha synuclein self-assembly process at intermediate stages during the process [55]. Figure 2.3a shows small oligomeric particles, figure 2.3b shows structures with more defined shapes called protofibrils and
figure 2.3c shows the fully formed fibrils. These TEM samples were prepared by negatively staining the protein solution with 2% uranyl acetate solution for 1 min.

Figure 2.3. TEM images of alpha synuclein at (a) 12 h (b) 36 h and (c) 96 h of incubation during the fibrillization process.
CHAPTER 3. SYNTHESIS AND CHARACTERIZATION OF METALLIC NANOWIRES ON ALPHA SYNUCLEIN TEMPLATE

3.1 Introduction

The synthesis of 1D structures using the bottom up approach was first reported by Braun and co-workers in 1998 [56]. The biotemplate that was used in this case for the fabrication of silver nanowires was DNA. The deposition of silver on DNA was carried out by a simple one step reduction method. This technique is also called the electroless deposition method. The biotemplated silver nanowire was later functionalized and an I-V measurement was obtained from this nanowire. After the first report of biotemplated synthesis of 1D structures, there were several reports in the literature describing the biotemplated synthesis of silver [56], gold [57], palladium [58, 59], and platinum [60, 61]. The increasing interest in the bottom strategy was due to the simple experimental approach, relatively inexpensive precursors, very short experimental time, mild experimental conditions and the biorecognition capacity of the biotemplate.

The 1D structures fabricated by the top down approach often required relatively complex experimental conditions [1-4]. Moreover the greatest challenge with the synthesized nanowires was that of placing the nanowires, in particular positions, in complex electronic circuits. This problem could be overcome by the bottom up technique
used for synthesizing nanowires. The biorecognition capacity of the biotemplate can be exploited to place the nanowires in precise locations in electronic circuits.

There have been several reports on the synthesis of metallic nanowires on DNA. Since the biomolecule is well studied and documented, the precise location of the amino acids and the charge present on the template are well known. This facilitates the successful functionalization of DNA and the synthesized nanowire can be placed in any desired position. There have been other biotemplates such as viruses [62] and microtubules [63-65] that have been used in the bottom up strategies, but proteins and amyloid fibers have been less explored. In the present work, amyloid like fibers has been used, as templates, for the fabrication of metallic nanowires.

3.2 Fabrication of Metallic Nanowires on Alpha Synuclein Template

The following paragraphs address the synthesis method, used for the preparation of metallic nanowires, and the obtained results. The alpha synuclein protein fibrils were used as a template for the fabrication of silver and platinum nanowires. The chemicals used in the metallization process were silver nitrate (AgNO₃) (0.1mM), potassium tetrachloroplatinate (K₂PtCl₄) (0.5mM), and sodium borohydride (NaBH₄) (0.003 wt %). A p-type Si(111) wafer was used as a substrate for the metallization process. Silicon was chosen as the substrate material for the synthesis of the nanowires due to its projected application in nanoscale electronic devices. Another substrate used for the metallization process was a 3-mm continuous carbon coated copper TEM grid. Figure 3.1 shows a schematic representation of the fabrication process.
A solution of α-synuclein protein fibrils in phosphate buffer (10μl) was pipetted onto the silicon substrate. The initial pH of the AgNO₃ solution was 5.9. In the next step, 10 μL of the silver nitrate solution was pipetted onto the silicon substrate and held for 15 minutes in atmospheric conditions. During this incubation, the Ag⁺ cations attach to the negatively charged groups of α-synuclein protein fibril. After incubation, 10 μL of the NaBH₄ solution was added to the previously held AgNO₃ solution and was kept on the substrate for 10 minutes. This reduced the Ag⁺ cations to metallic Ag(0). Finally, the substrate was rinsed with deionized water and dried under a jet of air. For the growth of platinum nanowires on the same template, a similar experimental procedure was used with the difference of the type of the metal ion solution (K₂PtCl₄), and the concentrations of the K₂PtCl₄ and NaBH₄ solutions. Briefly, 0.5mM K₂PtCl₄ solution was used as Pt²⁺ source, and 0.05 wt. % NaBH₄ was used as the reducing agent [55]. The α-synuclein fibrils were pipetted on to the substrate, to this K₂PtCl₄ was added and
incubated for 15 min. In the next step, NaBH$_4$ was pipetted on to the substrate and held for 10 min. The substrate was rinsed with deionized water and dried under a jet of air.

3.3 Results and Discussion

After metallization the diameter of the wires increased, indicating metal deposition on the fiber surface. Field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) were used to determine the diameter and physical morphology of the nanowires. Figures 3.2a and 3.2b show the FESEM images of silver (Ag) and platinum (Pt) nanowires, respectively. The average diameter of both Ag and Pt nanowires was in the range of 40-50 nm and their length varied between 500 nm and 1$\mu$m.

Figure 3.2. FESEM image of (a) a silver nanowire, with an average diameter of ~40nm and (b) a platinum nanowire, with an average diameter of ~50nm.

X-ray photoelectron spectroscopy (XPS) was used to verify the presence of specific elemental metals on the surface of the substrate. XPS was performed on the
silver nanowires synthesized on the silicon substrate. Figure 3.3 shows the XPS spectrum of a silver nanowire sample, with the binding energies of Ag3d_5/2 and Ag3d_3/2 at 367.83 and 373.83 eV, respectively, which are the typical values of Ag(0) [66]. The inset shows another XPS spectrum of the same sample with the peaks of Ag3s, Ag3p_1/2 and Ag3p_3/2. The result indicates the presence of silver on the substrate.

![XPS spectra of a silver nanowire sample on silicon substrate](image)

Figure 3.3. XPS spectra of a silver nanowire sample on silicon substrate. The spectra confirm the presence of silver on the substrate.

High resolution transmission electron microscopy (HRTEM) was used to verify the composition and nanocrystallinity of the synthesized nanowires. Figure 3.4 shows TEM images of the Ag nanowires, taken at different magnifications ranging from a medium to a high magnification where lattice fringes become visible. The high-resolution transmission electron micrograph (HRTEM) in Figure 3.4d, demonstrates the
crystallinity of the nanocrystals by the presence of lattice fringes. The Fourier-filtered image in Figure 3.4e, shows one of the two 200 planes, with a measured spacing of 0.204 nm, which is attributed to silver.

Figure 3.4. (a) Medium magnification bright field image of a typical nanowire. The highlighted region indicates the area shown in (b); (b) Higher magnification bright field image, showing clearly that the nanowires are composed of small particles. (c) HRTEM image demonstrating the crystallinity of the nanocrystals. (d) Selected HRTEM image of a well oriented region of one of the crystals (e) Fourier-filtered image, based on masking two 200 diffraction spots obtained from a diffractogram taken from (d). One of the two 200 lattice planes is indicated. The measured spacing of 0.204 nm is consistent with the crystals being silver.

Ultraviolet-visible (UV-Vis) spectroscopy technique was used to detect the presence of 1D metallic nanoscale structures [67, 68]. Specific signature peaks can be
used to extract information on the aspect ratio of the nanostructures along with the identification of the chemical elements that compose them. Moreover, the process of the variation in the nanowire diameter is correlated with the intensity of UV-Vis absorption peaks. To study the nanowire diameter variation as a function of reduction time by UV-Vis, the nanowire synthesis method described above was repeated in solution, without using a substrate. The concentration and pH conditions were similar to those used for nanowire synthesis on a substrate. UV-Vis spectra of the nanowire synthesis solution were registered at different reduction times, ranging between 5 and 45 minutes. Figure 3.5 shows the UV-Vis spectra of a silver nanowire sample with increasing reduction time. The spectra shows absorption peaks around 350nm and 380nm which indicate the presence of long silver nanowires with aspect ratios greater than 5 [67, 68]. The absorption peak at ~350 nm can be attributed to the plasmon resonance peak from the longitudinal vibrations in the silver nanowires, which is similar to that of bulk silver. The absorption peak at ~380 nm is due to the transverse plasmon vibration mode which indicates a very small dimension. These results confirm the presence of silver with a high aspect ratio. Furthermore, the absence of the absorption peak at ~410nm suggests that silver is not present in the colloidal form [67, 68]. Therefore, the results prove that the biotemplate synthesis on alpha synuclein fiber scaffolds led to the formation of Ag nanowires with aspect ratio greater than 5.
Figure 3.5. UV-Visible spectra of silver nanowire sample with increasing reducing time from 5 to 45 minutes. The peaks at 350 and 380 nm correspond to the presence of Ag in the form of nanowires. The reduction in the peak height as the reduction time increases shows a decrease in the density of nanowires in solution.

It can be observed from the UV-Vis spectra in Figure 3.5 that the absorption intensity decreases as the reduction time is increased. Moreover, there is a slight red shift in the position of the 380nm peak to 390nm with increasing reduction time. The reduction in the absorption intensity indicates a decrease in the density of nanowire and the shift in peak position suggests a nanowire diameter increase [67, 68]. In order to confirm the findings obtained from the UV-Vis experiment, similar synthesis was carried out on a TEM grid. Figure 3.6 shows TEM images of silver nanowire samples, synthesized with different reducing times. The diameter of the nanowires increased from 15 to 125nm with increasing reduction time from 5 to 35 minutes. The results indicate that a precise control of the reduction time during the synthesis leads to the formation of metallic nanowires with tunable diameters.
Figure 3.6. TEM images of silver nanowires with increasing reduction time of (a) 5, (b) 15, (c) 25, and (d) 35 minutes. The micrographs clearly show an increase in the diameter of the nanowire with increased reduction time.

A similar experiment was carried out to study the effects of the pH of the silver nitrate solution on the nanowire diameter, while keeping a constant reduction time of 10 minutes. The pH of the AgNO₃ solution was varied from 5.9 to 8.2 by the addition of ammonium hydroxide, keeping all other experimental parameters same as before. The diameter of the nanowires increased with the increase in pH. Figure 3.7 shows TEM images of silver nanowires with increasing pH values. Further TEM observations also showed that the density of the nanowires on the substrate decreased as the reduction time and pH increased. The decrease in the density of the nanowires with an increase in the
reduction time and the pH value, can be attributed to the agglomeration of thinner nanowires leading to the formation of thicker ones. This tendency is explained by the reduction in the total free energy of the system through the surface energy minimization criterion, while the total volume energy of the system remains the same.

A similar set of experiments were carried out to control the diameter of Pt nanowires. The results indicated the same trends in nanowire diameter variation as a function of reduction time and pH as for the synthesis of Ag nanowires.

Figure 3.7. TEM images of silver nanowires with increasing pH of (a) 5.9, (b) 6.7, and (c) 8.2. The results show an increase of the diameter of the nanowire as the pH of the salt solution increases.
To examine the continuity and metallic nature of the nanowires a two terminal I-V (current-voltage) curve was recorded for Pt nanowires. Platinum contacts were made to the nanowire by the focused ion beam (FIB) technique [69]. Figure 3.8 shows a FESEM image of a platinum nanowire with contacts, and the inset shows the nanowire morphology at a higher magnification. The two terminal I-V curve of the nanowire was linear with a measured resistance of ~60 kΩ. The breakage characteristic of the nanowire, under high voltage, was also studied. A high voltage, of 20V, was applied across the entire circuit and the heat generated due to the power loss (I²R) resulted in the melting of the nanowire. The small diameter of the nanowire (~ 40 nm) resulted in a very high resistance. This high resistance led to the production of a high amount of Joule heating that resulted in breaking the nanowire continuity. The discontinuity in the nanowire led the current to flow through the silicon substrate. The total series resistance increased to ~200KΩ and the corresponding I-V curve exhibited a slight deviation from linearity due to the semiconducting nature of the substrate. The I-V curves, before and after the nanowire breakage, are shown in figure 3.9. A clear change in the total series resistance due to the change in the current path can be observed. The I-V curve indicates that the nanowire was continuous and its linear dependence indicates the metallic nature of the nanowire. Figure 3.10 shows FESEM images of the platinum nanowire before and after breakage. The melted region can be clearly seen from the images and is expected to have had the smallest diameter which led to maximum local heating and thus breakage.
Figure 3.8. FESEM image of a platinum nanowire with contacts. The inset shows the same platinum nanowire at higher magnification.

Figure 3.9. Two terminal I-V curve of a platinum nanowire before and after nanowire breakage.
Figure 3.10. FESEM images of the platinum nanowire (a) before and (b) after breakage.

The nanowires synthesized by using DNA as the template appeared to have a similar morphology to that of the above synthesized nanowires. Several research groups such as Braun et al [56], Richter et al [69], Matsui et al [12] etc reported similar morphology of the nanowires. It was also reported that the shape of the nanowires was difficult to control and appeared to be a chain of nanoparticles. These synthesized nanowires were continuous and that was evident from the electrical measurements obtained from the nanowire.

3.4 Conclusion

In conclusion, the alpha synuclein template was successfully metalized. The synthesized silver and platinum nanowires were characterized by XPS, HRTEM and UV-Vis. The XPS data confirms the formation of silver on the substrate. The HRTEM images indicate the formation of 1D structures, composed of silver, on the alpha synuclein template. The diameter of the silver nanowires was varied by varying the process
variables, such as the reducing time and the pH of the salt solution. The diameter variation was deduced from the UV-Vis data and further confirmed from the TEM images. The platinum nanowires were used for electrical characterization. A two terminal I-V curve was obtained from a platinum nanowire. The electrical characterization data indicated that the platinum nanowire was continuous and conductive.

From the results obtained, it could be further concluded that amyloid like fibers could be used as biotemplates for the fabrication of 1D structures. The simple, inexpensive and mild experimental conditions make the synthesis process advantageous over other synthesis routes. Moreover, the control over the lateral dimensions helps in tuning the properties of the synthesized 1D structures. After the successful synthesis of diameter controlled metallic nanowires on the alpha synuclein template, the next step in the project was to design a similar experiment protocol for the fabrication of biotemplated semiconducting nanowires.
CHAPTER 4. SYNTHESIS AND CHARACTERIZATION OF SEMICONDUCTING NANOWIRES ON ALPHA SYNUCLEIN TEMPLATE

4.1 Introduction

The synthesis and characterization of semiconducting 1D structures have gained considerable attention in the past decade, due to the unique properties exhibited by these structures. Several synthesis techniques have been reported in literature for the fabrication of these 1D structures. The synthesis routes such as solvothermal process [70], thermal evaporation [71, 72], liquid crystal template [73] and electrodeposition [74] in porous anodic alumina templates are often used. These methods require high temperatures or pressures and a precise control of the process variables.

The bottom up strategy used for the fabrication of nanoarchitectures has also gained much attention due to the use of mild synthesis conditions and several other advantages as mention in the previous chapter. The synthesis of cadmium sulfide (CdS), lead sulfide (PbS) and zinc sulfide (ZnS) have been performed on DNA and other viral templates [62-65]. However, the amyloid like fibers have not been much explored. In the present chapter, the synthesis and characterization of CdS, PbS and ZnS on alpha synuclein template has been addressed. Semiconductor 1D materials such as CdS, PbS and ZnS are of considerable interest due to their proposed applications in optoelectronic, electronic devices and solar cells [27-29]. CdS as a potential semiconductor material has
received much attention because it is a direct bandgap semiconductor (2.42 eV), with its wavelength in the visible region. Various CdS nanostructures such as nanowires, nanotubes and quantum dots are being widely investigated for applications in nonlinear optical devices, photovoltaic cells and thin film transistors (TFTs) [77-79].

4.2 Fabrication of Semiconducting Nanowires on Alpha Synuclein Template

The following paragraphs address the synthesis method used for the fabrication of semiconducting nanowires, and the obtained results.

The alpha synuclein protein fibrils were used as a template for the fabrication of cadmium sulfide (CdS) and lead sulfide (PbS) nanowires. These nanowires were synthesized by using cadmium chloride (CdCl₂) (0.8mM) or lead nitrate (Pb(NO₃)₂) (0.5mM) as the salt solutions and hydrogen sulfide (H₂S) gas as the sulfur source. A p-type silicon (Si) (111) wafer was used as a substrate for the synthesis of CdS and PbS nanowires. The synthesis procedure was also repeated using a 3 mm diameter carbon coated gold grid as a substrate. Figure 4.1 shows a schematic representation of the fabrication process.
Figure 4.1. Schematic representation of the synthesis process used for the fabrication of semiconducting nanowires.

A volume of 10μl of alpha synuclein fibrils suspended in a phosphate buffer was pipetted onto the substrate before the addition of 10μl of CdCl₂ solution. The cadmium chloride solution was incubated with the alpha synuclein protein fibrils for 10 min. The solution was then exposed to H₂S gas, passed over the Si substrate for up to 2 min. The CdS containing substrate was rinsed with deionized water and dried under a jet of air. For the synthesis of PbS nanowires a similar experimental procedure was followed, using Pb(NO₃)₂ as the lead source and H₂S gas as the sulfur source [80].

4.3 Results and Discussion

Before the formation of CdS and PbS nanowires, the alpha synuclein fibrils appeared to have a uniform diameter. After the synthesis of CdS and PbS nanowires, the diameter of the fibril increased, indicating the deposition of CdS and PbS respectively. FESEM and TEM were used to determine the diameter and physical morphology of the
nanowires. Figures 4.2(a) and 4.2(b) show FESEM images of CdS and PbS nanowires, respectively. The average diameter of both nanowires was in the range of 40-50 nm and their length varied between 500 nm and 1 μm.

Figure 4.2. (a) FESEM image of a CdS nanowire, with an average diameter of ~40 nm and (b) a PbS nanowire, with a average diameter of ~50 nm.

HRTEM was used to verify the nanocrystallinity of the CdS and PbS nanowires. The figure 4.3 shows HRTEM images of the PbS and CdS nanowires, taken at high magnification, where lattice fringes become visible. The HRTEM images indicate that nanoparticles formed along the length of the protein fibril. The nanocrystals appear to be in close contact and overlapping with a diameter of 3-6 nm [80]. The HRTEM images in figure 4.3, demonstrate the crystallinity of the nanoparticles by the presence of lattice fringes.
Figure 4.3. (a) HRTEM image of a typical CdS nanowire. (b) HRTEM image of a typical PbS nanowire. The images in the inset are zoomed in images showing individual nanocrystals.

TEM bright field images and selected area diffraction (SAD) patterns were taken for each sample, in an effort to identify their crystal structures (Figure 4.4). The diffraction pattern from a CdS nanowire sample revealed the zinc blende structure of CdS.
Figure 4.4. Bright field TEM image of a CdS nanowire, and corresponding diffraction pattern (inset).

Similarly, bright field TEM images and corresponding diffraction patterns of PbS nanowire sample were recorded. The diffraction pattern was indexed and revealed a rock salt structure of PbS. Figure 4.5 shows the TEM bright field image and diffraction pattern (inset) obtained from the PbS sample.
In addition to the selected area diffraction (SAD) results, the presence of cadmium and sulfur in a CdS nanowires, and lead and sulfur in a PbS nanowires was confirmed using electron energy loss spectroscopy (EELS). Figure 4.6(a) shows the cadmium M_{4,5} edge at 404 eV [27] and figure 4.6(b) shows the sulfur L_{2,3} edge at 165 eV. Hence figure 4.6 clearly confirms the presence of cadmium and sulfur in a CdS nanowire. Figure 4.7 shows the lead O_{2,3} edge at 86eV and the sulfur L_{2,3} edge at 165eV. These EELS spectra in figure 4.7 also confirm the presence of lead and sulfur in PbS nanowire.
Figure 4.6. Electron Energy Loss Spectroscopy of CdS nanowires. The spectrum shows (a) the M$_{4,5}$ cadmium and (b) L$_{2,3}$ sulfur edges.
Figure 4.7. Electron Energy Loss Spectroscopy of PbS nanowires. The spectrum shows the presence of the O\textsubscript{2,3} lead edge (a) and of the L\textsubscript{2,3} sulfur edge (b).

Absorption spectrum was obtained from a CdS nanowire sample, to demonstrate its photoluminescent properties. Figure 4.8 shows absorption spectra obtained from a CdS nanowire sample, CdS colloidal sample and from alpha synuclein fibrils. The CdS
nanowire sample shows an absorption peak at 465 nm. The absorption peak of a colloidal sample was obtained at 485 nm [81, 82].

![UV Vis absorption spectra](image)

Figure 4.8. UV Vis absorption spectra obtained from CdS nanowires, CdS colloidal sample and from alpha synuclein fibrils. The inset shows the absorption peak of CdS nanowire for clarity.

The shift in the absorption peak could be due to the nanocrystal size in the biotemplate nanowires. In order to compare the particle size the above UV-Vis samples, CdS nanowires and CdS colloidal suspension, were prepared on a TEM grid. Figure 4.9 shows two TEM samples of CdS nanowire and CdS colloidal suspension samples for particle size comparison. From the TEM images it was evident that the change in the particle size had contributed to the shift in the absorption peak. The nanocrystal size in the CdS nanowire was ~2 – 6 nm, while the particle size in the CdS colloidal suspension was ~80 nm.
Figure 4.9. TEM image of CdS nanowire (a). The nanocrystal size was ~2 – 6 nm. TEM image of CdS colloidal suspension (b). The particle size was ~80 nm.
Nanowire synthesis experiments for both CdS and PbS were performed in an attempt to control the lateral dimension of the nanowires. A set of CdS and PbS nanowires was prepared by varying the time of H₂S gas exposure and by varying the pH of the salt solutions. It was observed that the diameter of the nanowires increased with increase in the H₂S exposure time and the pH of the salt solution. Figure 4.10 shows TEM images of CdS nanowires with increasing diameter as the pH of the salt solution was increased for a fixed exposure time of 2 min. The diameter of the nanoparticles increased from ~50 nm to ~100 nm with increasing pH. Figure 4.11 shows another set of TEM images with increasing nanowire diameter as the pH value was increased for a fixed exposure time of 6 min. The diameter of the particles increased from ~250 nm to ~350 nm with increasing pH.

Figure 4.10. TEM images of CdS nanowires after 2 min of H₂S gas exposure and CdCl₂ solution with a pH of (a) 5.9 and (b) 6.5.
Figure 4.11. TEM images of CdS nanowires after 6 min of H₂S gas exposure and CdCl₂ solution with a pH of (a) 5.9 and (b) 6.5

A similar set of experiment was performed to obtain PbS nanowires with varying diameter. The diameter could be varied in the range of 50 – 350 nm. Figure 4.12 and 4.13 below shows TEM images of PbS nanowires with increasing diameter as the pH of the salt solution was increased at a fixed exposure time of 2 min and 6 min respectively.

Figure 4.12. TEM images of PbS nanowires after 2 min of H₂S gas exposure time and Pb(NO₃)₂ solution with a pH of (a) 5.4 and (b) 6.5
The diameter of the semiconductor nanowires can be tuned by controlling the time of exposure to H$_2$S gas of the protein fibril incubated with lead or cadmium salts, and by adjusting the pH of the salt solution during synthesis. The cations remain in close proximity to the fibril surface in the initial step due to the electrostatic interaction, and subsequently they react with the S$^{2-}$ anions in H$_2$S leading to the formation of semiconducting nanoparticles that uniformly covered alpha synuclein fibrils.

In the next part of the project, the effect of incubation temperature on the morphology of the nanowires was investigated.

### 4.4 Fabrication of Zinc Sulfide Nanowires to Study the Effect of Incubation Temperature on Nanowire Morphology.

ZnS is a very interesting semiconducting material due to its wide range of proposed applications. It is an II-VI semiconducting material having a band gap of 3.7 eV. ZnS shows promise in several fields and has applications in electronics and
photonics. It has semiconducting, photoluminescent and field emission properties. These properties have been exploited in many applications such as light converting electrodes, ultraviolet light-emitting diodes, phosphors in cathode ray tubes, flat panel displays, injection lasers and infrared windows [83-85]. Several ZnS 1D structures such as nanorods, nanowires, nanobelts and nanotubes have been fabricated [86-88]. All these structures have been synthesized at high temperatures and require long reaction times.

The bottom up synthesis technique was used in the present work for the fabrication of ZnS nanowires on alpha synuclein template. The synthesis conditions were relatively mild and the effect of incubation temperature on the morphology of the nanowires was studied.

The synthesis of zinc sulfide (ZnS) nanoparticle chains was carried out by using zinc chloride (ZnCl₂) (2 mM) as the salt solution, and hydrogen sulfide (H₂S) gas as the sulfur source. A stock solution of ZnCl₂ was prepared and its pH value was adjusted to be in the acidic regime by the addition of concentrated hydrochloric acid. This was done to avoid precipitation of zinc hydroxide in solution. For the synthesis of ZnS nanoparticle chains a p-type silicon (Si) (111) wafer was used as a substrate to prepare a FESEM sample. The same synthesis procedure was performed on a 3 mm diameter carbon coated gold grid as a substrate to obtain a TEM sample. A volume of 10μl of alpha synuclein fibrils suspended in the PBS buffer was pipetted onto the, Si wafer, substrate. The ZnCl₂ solution (10μl) was deposited on to the protein solution, followed by an incubation time of 10 min. The substrate with the protein and the ZnCl₂ solution was then exposed to H₂S gas for 2 min. Later, the substrate was rinsed using deionized water and dried under a jet of air. A similar procedure was carried out for the preparation of a TEM sample.
The morphology and average diameter of the nanowires was obtained after analyzing the FESEM and TEM images of the ZnS samples. Figures 4.14a and 4.14b show one FESEM and one TEM image, respectively, of ZnS nanowires. The average diameter for these samples was approximately in the range of 60-65 nm. The inset in the TEM image shows the alpha synuclein template between the two ZnS nanoparticles, possibly stained by the metal salt.

Figure 4.14. FESEM image of ZnS nanowires (a). TEM image of ZnS nanowire (b). The inset is a zoomed in image of the highlighted area showing a possible exposure of the alpha synuclein fibril, lightly stained by ZnS, between two ZnS nanoparticles

HRTEM imaging was carried out on the ZnS nanowires, to study the nanocrystalline nature of the samples. The HRTEM images indicate that the nanoparticles are composed of several nanocrystals which have an approximate dimension of ~2 nm. The figure 4.15a is a HRTEM image of a ZnS sample. The lattice fringes are clearly visible, which indicate the nanocrystalline nature of the ZnS sample, in the images shown in figure 4.15b and 4.15c. The images in figure 4.15b and 4.15c are zoomed-in images of the ZnS nanoparticle chain for clear representation of the lattice fringes.
Figure 4.15. HRTEM image (a) of ZnS nanoparticle chains. Images in b and c are zoomed in images of the highlighted regions of image (a), showing the lattice fringes.

A TEM bright field image and a selected area diffraction (SAD) pattern were obtained from one of the ZnS nanowires for crystal structure investigations. Figure 4.16 shows a bright field image of a ZnS nanowire and the inset shows the diffraction pattern obtained. The SAD pattern indicates the formation of zinc blende structure.
Along with the SAD results, the presence of Zn and S in ZnS nanowires was ascertained by performing EELS on the ZnS samples. The EELS spectra obtained from the ZnS sample are shown in figure 4.17(a) and figure 4.17(b). The figure 4.17(a) shows the Zn L₃, L₂ edges at 1020 and 1043 eV. The Zn L₁ edge at 1194 eV is also clearly visible, even though it is minor edge. Figure 4.17(b) shows the sulfur L₂,3 edge at 165 eV. These spectra confirm the presence of Zn and S in the sample, further supporting the SAD results.
Figure 4.17. Background subtracted EELS spectra obtained from ZnS nanowires, showing (a) the Zn L\textsubscript{3}, L\textsubscript{2}, and L\textsubscript{1} edges at 1020, 1043, and 1194 eV and (b) the S L\textsubscript{2,3} edge at 165 eV.

Elemental mapping using the Zn L\textsubscript{3} edge at 1020 eV and the S L\textsubscript{2,3} edge at 165 eV, respectively, was also performed on one of the ZnS nanowires to study the distribution of Zn and S in the nanowires. The figure 4.18(a) shows a zero energy loss...
image, followed by the sulfur map (4.18b) and zinc map (4.18c). These elemental maps show that Zn and S are uniformly distributed through the whole nanowire.

![Elemental Maps](image.png)

Figure 4.18. A Zero energy loss image (a), (b) sulfur map using L₂,₃ edge at 165 eV, and (c) zinc map using L₃ edge at 1020 eV

The next step in the characterization of the ZnS nanowires was to perform UV-visible (UV-Vis) spectroscopy on the ZnS nanowire sample and on ZnS colloidal sample having a particle size of 10μm, purchased from Sigma Aldrich for comparison of the absorption peaks. Figure 4.19 shows two UV-Vis spectra obtained from ZnS nanowires (a) and ZnS powder (b). The spectrum obtained from the ZnS nanowire sample showed an absorption peak at ~310 nm. The absorption peak for the colloidal ZnS sample was obtained at ~345 nm. The absorption peak at ~310 nm for the ZnS nanowire appears to have blue shifted [30]. This shift is consistent with the quantum confinement effect. The UV-Vis results were confirmed with the help of HRTEM images obtained from the ZnS nanowire sample. The HRTEM images indicate that the nanowires are composed of nanocrystals, having a dimension of ~2 nm (20Å).
Figure 4.19 UV-Vis spectra obtained from a ZnS nanowire sample. (a) An absorption peak at ~310 nm corresponds to the ZnS nanowire. The next absorption spectrum (b) was obtained from the colloidal ZnS sample, showing an absorption spectrum of ~345 nm.

Further, in an attempt to achieve control of the nanowire diameter a series of samples were prepared with varying process conditions similar to the experiments carried out for CdS and PbS. It was observed that the nanowire diameter increased with increase in the H2S exposure time of 2, 5 and 10min. Similar results were obtained with the variation of the pH of the salt solution. The pH values used in the experiment was 4, 5 and 6. Figure 4.20 and 4.21 show variation in the diameter of the ZnS nanowires with increase in the exposure time and pH of the salt solution respectively. The diameter could be varied in the range of 30 – 165 nm.
Figure 4.20. TEM images of ZnS nanowires obtained after (a) 2 min, (b) 5 min and (c) 10 min of H₂S gas exposure.
Figure 4.21. TEM images of ZnS nanowires obtained for different pH values of the salt solution (a) pH 4, (b) pH 5 and (c) pH 6

In an additional experiment, the effect of incubation temperature of the salt solution on the morphology of the nanowires was studied (The previous studies described above were carried out at 22°C). Here, two separate experiments were carried out where the salt solution was heated to a temperature of 45°C and 85°C for 30min and then used in the synthesis process. The pH value of the salt solution was kept at 5 and the H₂S gas exposure time was fixed to 2 min. Figure 4.22 shows a TEM image of a ZnS nanowire obtained by using a ZnCl₂ solution at 45°C. When the ZnS nanowire obtained at 45°C were compared with the ZnS nanowire obtained by varying the H₂S exposure time (Figure 4.20a) and the pH of the salt solution (Figure 4.21b), it was observed that the ZnS nanoparticles, obtained at 45°C, appeared to be slightly more connected. Figure 4.22 has
several highlighted regions (also shown magnified in the inset). The left two zoomed-in images show regions where the alpha synuclein template can be seen. Although the ZnS nanoparticles look very well connected, there are a few regions along the length of the nanowire that look disconnected thus exposing the alpha synuclein template. However, there are many other regions that clearly show the formation of the neck between the ZnS nanoparticles (shown in the inset to the right).

Figure 4.22. TEM image of a ZnS nanowire obtained at 45°C. The insets in the image are zoomed-in images of the highlighted regions. The left two insets show the alpha synuclein template between the ZnS nanoparticles. The inset to the right shows the formation of neck regions between the nanoparticles.

To improve the connectivity of the ZnS nanowires, the ZnCl₂ salt solution was heated to a temperature of 85°C for 30 min prior to its use in the synthesis process. The
other variables used in the synthesis process were kept constant, for a better comparison with the sample obtained at 45°C. Figure 4.23 is a TEM image of a ZnS nanowire obtained at 85°C. When compared with figure 4.23, it can be clearly seen that the nanoparticles are well connected and there are no regions where the alpha synuclein template can be seen. The neck regions that can be seen between ZnS nanoparticles are shown in the inset for clarity. The neck regions look very well defined. Thus by varying the incubation temperature the connectivity between the ZnS nanoparticles can be improved.

The changes in the process variables help in varying the size of the nanowire and also help in varying the connectivity between the nanoparticles, thus making the nanoparticles more smoothly connected.
Figure 4.23. A TEM image of a ZnS nanowire. The insets are zoomed-in images of the highlighted regions. The inset clearly shows well developed neck regions between ZnS nanoparticles

4.5 Conclusion

In summary, semiconducting nanowires such as CdS, PbS and ZnS were successfully synthesized on the alpha synuclein template. The nanowires were characterized by HRTEM, EELS and UV-Vis. The EELS data confirmed the formation of CdS, PbS and ZnS nanowires. The EELS spectra distinctively showed Cd, Pb, Zn and S edges. The UV-Vis data indicated that the particle size in the nanowire sample was much smaller in dimension as compared to the colloidal samples prepared in the absence of the alpha synuclein template. The HRTEM images suggested a nanocrystalline nature
of the nanowires. The size of the nanocrystals was in the range of 2 – 6 nm. The
diffraction pattern revealed a zinc blende structure of CdS and ZnS, and a rocksalt
structure of PbS. The diameter of the nanowires was varied by varying the process
parameters, such as H₂S exposure time and the pH of the salt solution. The incubation
temperature helped to improve the coverage of the alpha synuclein template in the case of
ZnS nanowires.

From the above results, it could be concluded that the amyloid like fibers can be
used as templates for the synthesis of semiconducting 1D structures. The nature of the
experimental conditions, the surface charge on the template, controlled reaction time and
room temperature synthesis, helps to synthesize extremely small dimensions of
nanocrystals (~2 nm) on the alpha synuclein template. Due to these small nanocrystals
the synthesized structures could exhibit improved properties when compared with their
bulk counterparts
CHAPTER 5. NATURAL BIOPOLYMERS AS TEMPLATES FOR METALLIC AND SEMICONDUCTING NANOSTRUCTURE SYNTHESIS.

5.1 Introduction

Fabrication of nanoparticles has attracted much attention in the past decade because of their potential in the development of various technologies, such as in sensing or in catalysis. Metallic nanoparticles incorporated into polymers in particular attracted interest due to the capacity of the nanoparticle to impart their unique properties to these polymers. Majority of the metal nanoparticles containing polymers were prepared by reduction of metal salts on polymer templates [89, 90], or UV radiation [100]. The size, dispersity, structure and chemical properties of the metallic nanoparticles are strongly influencing the properties of these nanocomposites. Polymer nanofibers can be used for wound dressing, as separation filters, scaffolds for tissue engineering, or in sensing, mostly due to their high surface area, chemical and biological properties and sometimes high porosity [100]. Fabrication of synthetic polymers often involves either complicated and expensive procedures, or toxic chemicals. On the other hand, natural fibers are abundant in nature in plants, such as forest products, grasses, reeds or stalks. Cellulose is the main natural polymer widely available from these natural sources and can be a viable alternative to synthetic polymers or even inorganic reinforcing fibers with applications in composite materials. Cellulose fibers are inexpensive, renewable, abundantly available in
variety of forms, and display a high chemical reactivity of their surface due to the free hydroxyl groups that can be further used for chemical modification. Other biomolecular templates such as DNA, microtubules, beta amyloids, the yeast prion protein Sup35NM, tobacco mosaic virus and alpha synuclein, have been used for the template synthesis of nanomaterials from the bottom-up [55, 62-64, 80, 115]. The bio-templated self-assembly, takes advantage of the nanoscale dimensions and the surface charge of various peptides, proteins and DNA. However, these templates are more expensive and complicated to produce, and can find less applicability than cellulose nanocrystal. Developing a simple method to decorate cellulose nanocrystals with metallic nanoparticles, which takes advantage of the natural properties of cellulose and enables precise control over the nanoparticle dimensions is therefore of a significant scientific interest. Previous work in the area include synthesis of silver nanoparticles on cellulose acetate nanofibers [100], stabilization of gold-silver nanoparticles on cellulose nanocrystals, or deposition of metal nanoparticles into various porous cellulosic matrices [89, 99, 101]. However, most of these reports show either random deposition into a porous matrix, or a rather low coverage of nanoparticles on the cellulose. In the present work, higher density of nanoparticles has been synthesized on the cellulose template. This was carried out by surface modification of the cellulose. Cellulose is an oxygen rich natural carbohydrate that consists of anhydroglucose units. These glucose units are joined together by an oxygen linkage to form a linear molecule. The structure of cellulose is shown in figure 5.1 below.
Figure 5.1. The structure of cellulose

Figure 5.2 shows two TEM images of the cellulose fiber. The image (a) is an unstained TEM image while image (b) is the stained TEM image. The fiber is ~10 nm in diameter and the length ranges from 1 micron to several microns. Moreover, the cellulose fiber is resistant to the electron beam and does not suffer beam damage as other proteins or viruses.

Figure 5.2. TEM images of the cellulose fiber. (a) An unstained TEM image. (b) A stained TEM image

The following paragraphs address the synthesis of metallic nanoparticles on cellulose via surface modification of the template.
5.2 Fabrication of Metallic Nanoparticles on the Cellulose Template

The chemicals used in the synthesis of metallic nanoparticles were silver nitrate (AgNO₃), copper chloride (CuCl₂), hydrogen tetrachloroaurate (HAuCl₄) and potassium tetrachloroplatinate (K₂PtCl₄) as the metal precursors. The reducing agent used was sodium borohydride (NaBH₄). The surfactant used for the surface charge modification of cellulose template was cetyltrimethylammonium bromide (CTAB). All the samples were prepared on a carbon coated copper TEM grid. Figure 5.3 shows a schematic representation on the fabrication process for the synthesis of silver nanoparticles on the cellulose.

Figure 5.3. Schematic representation of the silver nanoparticle synthesis process.
A solution of cellulose suspended in distilled water (3 ul) was pipetted on a TEM grid. The surfactant CTAB (0.1 mM) (3 ul) was then pipetted on to the cellulose template and incubated for 5 min. This was followed by an addition of AgNO₃ (0.2 mM) solution (3 ul) and an incubation of 5 min. In the next step NaBH₄ (0.03 wt %) (3 ul) was added and held for 5 min. The substrate was washed with deionized water and dried in air. Similarly Cu, Au and Pt nanoparticles were synthesized on the cellulose template. Briefly, cellulose was treated with CTAB for 5 min followed by the addition of CuCl₂ (0.8 mM), HAuCl₄ (0.8 mM) or K₂PtCl₄ (0.5 mM) solution with an incubation time of 5 min. The reducing agent used was NaBH₄ (0.03 wt %) and the reducing time was kept at 5 min in each nanoparticle synthesis experiment.

5.3 Results and Discussion

In the case of Ag nanoparticle synthesis, before the synthesis process the cellulose template appeared uniform and smooth along the length (Figure 5.2). After the completion of the synthesis, small silver nanoparticles were stabilized on its surface. The particle size of these silver nanoparticles was measured by TEM. The average particles size was determined to be ~20 nm. Figure 5.4 shows two TEM images. The image (a) shows a cellulose fiber with two silver dendrites at each end of the fiber. This sample was prepared without the use of CTAB. Here the cellulose template was used without any surface charge modification. The image (b) shows cellulose fibers with silver nanoparticles decorated on the surface of the template. This sample was prepared in the
presence of CTAB. After comparing the TEM images it was concluded that CTAB helps in the formation of silver nanoparticles on the cellulose template.

Figure 5.4. TEM image of a cellulose fiber in the absence of CTAB (a) and in the presence of CTAB (b)

Although there was a drastic difference in the number of silver nanoparticles on cellulose, the coverage of the template was not uniform and complete. In order to improve the coverage of cellulose, the concentration of CTAB was varied. The fabrication process was followed as described above with varying concentrations, from 0.1 mM to 1 mM, of CTAB. Figure 5.5 shows four TEM images of silver nanoparticles on cellulose with varying concentrations of CTAB. From these images, it was observed that the nanoparticle coverage increased gradually from 0.1 mM (Figure 5.4a) to 0.5 mM (Figure 5.4c) and then decreased for 1 mM (Figure 5.4d) of CTAB. The low coverage of nanoparticles for 0.1 and 0.2 mM CTAB could be due to the lower amount of surfactant as compared to the cellulose concentration in the sample. The coverage of the template improved for 0.5 mM CTAB, this could be due to the optimum level reached between the surfactant concentration, precursor concentration and the cellulose concentration. Later,
the coverage was observed to decrease for 1 mM CTAB. The decrease in coverage could be due to the formation of rod like micellar structures of CTAB and lower amounts of CTAB available for the stabilization of the synthesized nanoparticles. Thus 0.5 mM was found to be the optimum concentration for the nanoparticle synthesis on cellulose. In order to obtain consistent results, all the remaining experiments were performed by keeping the CTAB concentration to 0.5 mM.

![Figure 5.5: TEM images of silver nanoparticles on cellulose with varying concentrations of CTAB, (a) 0.1mM, (b) 0.2mM, (c) 0.5mM and (d) 1mM](image)

To further characterize the silver nanoparticles, HRTEM imaging was performed. The HRTEM image in Figure 5.5 shows silver nanoparticles on cellulose. The cellulose template was not visible due to the low contrast resulting from the lighter elements (N, C, H and O) that make up the cellulose compared to the higher electron density of metallic
silver. The HRTEM image shows lattice fringes on the silver nanoparticles, indicating their crystalline nature. It was also observed that the silver nanoparticles were facetted, since the entire nanoparticle was not in focus (Figure 5.6). The silver nanoparticles have an average size of ~20 nm. The inset in Figure 5.6 clearly shows the lattice fringes of the silver nanoparticle and the interplanar spacing was found to be ~0.23 nm. The interplanar spacing matched well with two (111) planes of silver. The information garnered from the HRTEM image confirmed the presence of silver thus indicating the formation of silver on the cellulose surface. Further, the silver nanoparticles were crystalline and facetted in nature.
Figure 5.6. HRTEM image of a single silver nanoparticle on a cellulose template. The inset shows the interplanar spacing to be \( \sim 0.23 \) nm, which can be attributed to two 111 planes of silver.

To further confirm the formation of silver, electron energy loss (EELS) spectra were also obtained from a silver nanoparticle sample. Figure 5.7 shows a bright field TEM image (a) and an EELS spectrum (b) obtained from the same region shown in (a). The EELS spectrum very clearly shows a silver major edge at 367 eV. Since the 367 eV is a major edge of silver, it further confirms the formation of silver nanoparticles on cellulose.
Figure 5.7. TEM image (a) of a silver nanoparticle sample. It is also a region where EELS spectrum was obtained. (b) EELS spectrum of a silver nanoparticle sample showing an energy loss edge at 367eV.

During the fabrication process, the surfactant CTAB could help in stabilizing the synthesized nanoparticles and also could help in bonding the nanoparticles to the template. The surfactant CTAB has been used for the synthesis of several noble metal nanorods [102] and nanoparticles [103], since it has a tendency of forming micellar
structures that stabilize the synthesized nanostructures. Here in the fabrication of silver nanoparticles on cellulose, CTAB could be assisting to confine the size of the silver nanoparticles. Moreover, the hydrophilic part of the surfactant CTAB could interact with the hydroxyl groups of the cellulose, forming hydrogen bonds with the template. Thus the CTAB works to stabilize the synthesized nanoparticle and to connect it to the cellulose.

The possible interaction of CTAB with the cellulose surface leads to the formation of high density silver nanoparticles. The evidence of high density nanoparticle formation, in the presence of CTAB, is given in Figure 5.4. The figure 5.8 is a schematic representation of the formation process. The schematic elucidates the function of CTAB in the synthesis of silver nanoparticles on cellulose.

![Schematic representation of the formation process](https://example.com/schematic.png)

Figure 5.8. Schematic representation of the formation process, which explains the role of CTAB.
In order to obtain an optimum coverage of the cellulose and an optimum particle size, several sets of experiments were performed. As indicated above, the first step in this optimization process was to monitor the influence of the concentration of AgNO₃. The fabrication process was followed according to the description given above with varying concentrations, from 0.2 mM to 1 M, of AgNO₃. The samples were synthesized in the solution form and were characterized by the UV-Vis spectrometer. Briefly, the cellulose solution was taken in an Eppendorf tube. To this, CTAB (0.5 mM) was added and incubated for 5 min. AgNO₃ was added to this mixture of solutions and an incubation step of 5 min was followed. In the last step, NaBH₄, a reducing agent, was added and held for 5 min. The UV-Vis spectrum was obtained after the 5 minutes of reducing time. Figure 5.9 shows the changes in the UV-Vis spectra obtained from the silver nanoparticle sample with varying AgNO₃ concentration. It was observed that the absorption peak was centered at ~405 nm, which can be attributed to silver nanoparticles [104, 105]. By increasing the AgNO₃ concentration from 0.2 mM to 1 M, several observations could be made. The spectra for 0.2 mM, 10 mM and 100 mM showed an unchanged absorption peak at ~405 nm. It was also observed that the UV-Vis spectrum appeared to shift (figure 5.9 inset) from ~405 nm to ~425 nm for 1 M AgNO₃, due to the increase in the size of the silver particles as the concentration of AgNO₃ increased [89].
Figure 5.9. UV-Vis absorption spectrum of a silver nanoparticle sample showing an absorption peak at 405 nm. The inset shows the absorption peak at ~425 nm for 1M AgNO₃.

From the UV-Vis experiment it was evident that the particle size increased with increase in the concentration of the salt solution, AgNO₃ in this case. The nucleation of new Ag nanoparticles occurs simultaneously with the growth of other particles. Once the first Ag nuclei are formed, the colliding nuclei start growing, while a higher concentration of AgNO₃ leads to the formation of new nuclei. This mechanism is also supported by evidence of polydispersity of Ag nanoparticles.

Another set of experiments, were performed by varying the reaction time. The synthesis procedure was followed as described above with a variation of the reducing time from 2 to 30 minutes. The pH of the salt solution, AgNO₃ in this case, was at a value of 5.5. Figure 5.10 shows four TEM images of silver nanoparticle samples synthesized with varying reducing time. It was observed that the particle size increased, from ~17 nm to ~36 nm, with increase in the reducing time. Along with increase in the size of the nanoparticles, the coverage of cellulose appeared to have improved. Figures 5.10 (a) and
(b), when compared to Figures 5.10 (c) and (d) clearly showed fewer gaps between the nanoparticles.

Figure 5.10. TEM images of silver nanoparticle with varying reducing time, (a) 2 min, (b) 5 min, (c) 15 min and (d) 30 min.

In the next of experiments, the reducing time was kept constant at 5 min, and the pH of the salt solution was varied between 4.5 and 8.5. Figure 5.11 shows two TEM images of silver nanoparticles on cellulose with varying pH of AgNO₃. It was observed that the particle size increased with increase in the pH of the salt solution. It was also observed that the coverage of the template improved with an increase in the pH of the salt solution. However, the higher pH value sample lead to a large amount of non-specific
silver deposition on the substrate. These results are in agreement with other reports in literature [106] that indicate that at a basic pH, aggregation of Ag nanoparticles obtained by surfactant stabilization and NaBH₄ reduction occurs. This effect can be attributed to hydrophobic interactions between uncharged CTAB molecules at slightly basic pH, rendering them insoluble, i.e. unable to stabilize and prevent Ag nanoparticle aggregation.

Figure 5.11. TEM images of silver nanoparticle samples with varying pH of the salt solution (a) pH = 4.5 and (b) pH = 8.5

Along with the synthesis of silver nanoparticles, other metallic nanoparticles were also synthesized by using the same general procedure. Metallic nanoparticles, such as Cu, Au and Pt were fabricated on the surface of the cellulose template and were characterized by UV-Vis and TEM. The morphology, in general, of these metallic nanoparticles appeared to be quite similar to that of silver nanoparticles. Figure 5.12 (a) shows a bright field TEM image of Cu nanoparticle sample. The inset shows the zoomed in image of the highlighted region. Here, it was observed that the Cu nanoparticles were very fine and did not appear to have agglomerated. The average particle size was ~5 nm. The figure (b)
shows the UV-Vis absorption peak at ~600 nm indicating the formation of Cu nanoparticles [107, 108].

Figure 5.12. (a) TEM image of copper nanoparticles on a cellulose template. (b) An absorption spectrum obtained from a copper sample, showing an absorption peak at ~600 nm.

Figure 5.13 (a) shows a bright field TEM image of Au nanoparticles on cellulose. The inset in the figure shows a zoomed in image of the highlighted region. Here, it was
observed that the nanoparticles have agglomerated thus forming several and larger gaps along the cellulose. The figure 5.13 (b) is an UV-Vis absorption spectrum showing an absorption peak at ~535 nm, which can be attributed to Au nanoparticles [109, 110].

Figure 5.13. (a) TEM image of gold nanoparticles on cellulose. (b) An absorption spectrum obtained from a gold sample, showing an absorption peak at ~535 nm.

Pt nanoparticles were also fabricated on cellulose. Figure 5.14 (a) shows a bright field TEM image of platinum nanoparticles on cellulose. The inset shows a zoomed in image of the highlighted region. Here, it was observed that the nanoparticles were very
fine and uniformly placed along the length of the template. The average particle size was ~5 nm. The figure 5.14 (b) shows an UV-Vis spectrum from the platinum nanoparticle sample. The absorption peak is in the range of ~200 – 300 nm, indicating the formation of platinum nanoparticles [111, 112].

After successful completion of the synthesis of metallic nanoparticles on cellulose, this work was further extended to fabricate semiconducting nanoparticles on
the cellulose fiber. The following paragraphs address the synthesis and characterization of semiconducting nanoparticles on cellulose.

5.4 Fabrication of Semiconducting Nanoparticles on the Cellulose Template

The chemicals used in the synthesis of semiconducting nanoparticles were cadmium chloride (CdCl₂), lead nitrate (Pb(NO₃)₂), zinc chloride (ZnCl₂), hydrogen sulfide gas (H₂S) and cetyltrimethylammonium bromide (CTAB). The salt solutions were used as the cadmium (Cd), lead (Pb) and zinc (Zn) precursors respectively. The H₂S gas was used as the sulfur source. The surface charge of the cellulose was modified by CTAB. All the samples were prepared on a carbon coated copper TEM grid. Figure 5.15 shows a schematic representation on the fabrication process.
A solution of the cellulose fibers suspended in distilled water (3 ul) was pipetted on a TEM grid. The surfactant CTAB (0.5 mM) (3 ul) was then pipetted on to the cellulose and incubated for 5 min. This was followed by an addition of CdCl₂ (0.8 mM) solution (3 ul) and an incubation of 5 min. In the next step H₂S gas was made to pass over the substrate for 2 min. The substrate was washed with distilled water and dried in air.

Similarly PbS and ZnS nanoparticles were synthesized on the cellulose. Briefly, the cellulose was treated with CTAB for 5 min followed by the addition of Pb(NO₃)₂ (0.5 mM) or ZnCl₂ (2 mM) solution with an incubation time of 5 min. The H₂S gas was made to pass over the substrate for 2 min. in each nanoparticle synthesis experiment.
5.5 Results and Discussion

Fabrication of semiconducting nanoparticles onto the surface of a biological template can be achieved by various methods [UV, gamma irradiation] but the popular electroless deposition technique is often used due to its simplicity and mild synthesis conditions. Here, semiconducting nanoparticles (CdS, PbS and ZnS) on cellulose were synthesized using a modified electroless deposition method. The reductive deposition of metals, particularly silver in the presence of proteins and DNA was reported by Merrill [113, 114]. The driving force of the deposition reaction relies on the difference between the redox potentials of the biomolecules and those of the protein or DNA. Presently, the method is widely used for the detection of proteins and nuclein acids in silver stained gels.

A similar approach was used in coating biological templates with metals and semiconducting materials. However, this approach has limited success in the coating of cellulose. The reason for the difficulty in the direct application of the same approach to the cellulose template could be the surface charge of polysaccharides present on the cellulose template. The surface charge is mostly neutral, with no significant negative charges on its surface, unlike DNA or viruses like TMV. The nearly neutral charge on the cellulose template was modified by using CTAB to facilitate the synthesis of semiconducting nanoparticles on the cellulose template. In the later stage of the synthesis process, the CTAB stabilizes the semiconducting nanoparticles (CdS, PbS and ZnS) on the template. In the synthesis process, the process parameters that control the rate of reaction are H₂S exposure time, pH of the salt solution, concentration of the salt solution
and the incubation temperature of the salt solution. In order to control the rate of reaction the above parameters could be varied independently.

In the case of semiconducting nanoparticle synthesis, before the synthesis process the cellulose template appeared uniform and smooth along the length. After the completion of the synthesis, small semiconducting nanoparticles were stabilized on its surface. The particle size of these nanoparticles was measured by TEM. The average particles size was determined to be in the range of ~20 – 30 nm. Figure 5.16 shows two TEM images. The image (a) shows several cellulose fibers with the deposition of non-specific CdS on the substrate. This sample was prepared without the use of CTAB. Here the cellulose template was used without any surface charge modification. The image (b) shows CdS nanoparticles on cellulose fibers thus resulting in the formation of CdS nanowires on cellulose. This sample was prepared in the presence of CTAB. The comparison between the two TEM images concluded that CTAB helps in the synthesis of semiconducting nanoparticles, like metallic nanoparticles, on cellulose.

Figure 5.16. TEM image of several cellulose fibers and non-specific CdS nanoparticles, obtained in the absence of CTAB (a). The image (b) shows CdS nanoparticles on the cellulose template, synthesized in the presence of CTAB.
To characterize the synthesized semiconducting nanoparticles; UV-Vis spectra were recorded from the CdS and ZnS samples (Figure 5.22 and 5.23). The UV-Vis spectra showed an absorption peak at 465 nm and 310 nm for CdS and ZnS respectively. The absorption peaks matched well that of CdS and ZnS respectively [80, 115]. To confirm the results obtained from the UV-Vis spectra, electron energy loss spectra (EELS) were obtained from the CdS, PbS and ZnS samples. Figure 5.17 shows EELS spectra recorded from the CdS nanoparticle sample. The EELS spectra clearly show the Cd M\textsubscript{4,5} edge at 404 eV and the S L\textsubscript{2,3} edge at 165 eV. These spectra confirm the formation of CdS on the cellulose template.
Figure 5.17. EELS spectra obtained from CdS sample, showing the Cd M$_{4,5}$ edge (a) at 404 eV and the sulfur L$_{2,3}$ edge (b) at 165 eV.

Similarly EELS spectra were obtained from PbS and ZnS samples. Figure 5.18 shows EELS spectra obtained from PbS, which indicate the Pb O$_{2,3}$ edge at 86 eV and S L$_{2,3}$ edge at 165 eV. Thus confirming the formation of PbS on the cellulose template. The
EELS spectra obtained from the ZnS sample showed the Zn edges at 1020, 1043 and 1194 eV. The S edge was obtained at 165 eV.

Figure 5.18. EELS spectra obtained from PbS sample, showing the Pb O\textsubscript{2,3} edge (a) at 86 eV and the sulfur L\textsubscript{2,3} edge (b) at 165 eV.

To further characterize the semiconducting nanoparticles on the cellulose template, HRTEM imaging was performed. The HRTEM image in Figure 5.19 shows CdS nanoparticles on the cellulose template. The cellulose template was not visible due
to the low contrast of lighter elements (N, C, H and O) that make up the cellulose template compared to the semiconducting material. The HRTEM image shows lattice fringes on the CdS nanoparticles, indicating their crystalline nature. It was also observed that the CdS nanoparticles were in the size range of 2 – 6 nm (Figure 5.19). The information garnered from the HRTEM image confirmed the crystalline nature of the CdS nanoparticles on the cellulose template. Similar results were obtained for PbS and ZnS samples (shown in figure 5.20 and 5.21 respectively).

Figure 5.19. HRTEM image of CdS nanoparticles on the cellulose template. The image indicates the crystalline nature of the CdS on the cellulose template.
Figure 5.20. HRTEM image of PbS nanoparticles on the cellulose template. The image indicates the crystalline nature of the PbS on cellulose template.
Figure 5.21. HRTEM image of ZnS nanoparticles on the cellulose template. The image indicates the crystalline nature of the ZnS on cellulose template.

Here in the fabrication of semiconducting nanoparticles on the cellulose template, CTAB could also be assisting to confine the size of the semiconducting nanoparticles. The CTAB could act as a stabilizing agent during the formation of semiconducting nanoparticles. The hydrophilic end of the CTAB micelle then could interact with the hydroxyl group of the cellulose template by the formation of hydrogen bonds. Thus the CTAB connects the synthesized semiconducting nanoparticles to the cellulose template. This possible interaction of CTAB with the cellulose template surface leads to the
formation of high density semiconducting nanoparticles. The evidence of high density nanoparticle formation, in the presence of CTAB, is given in Figure 5.16.

In order to obtain an optimum coverage of cellulose and a variation in particle size, several sets of experiments were performed. The first step in this optimization process was to monitor the influence of the concentration of CdCl₂. The fabrication process was followed according to the description given above with varying concentrations, from 0.8 mM to 1 M, of CdCl₂. The samples were synthesized in the solution form and were characterized by the UV-Vis spectrometer. Briefly, cellulose suspended in distilled water was taken in an Eppendorf tube. To this, CTAB (0.5 mM) was added and incubated for 5 min. The CdCl₂ was added to this mixture of solutions and an incubation step of 5 min was followed. In the last step, H₂S gas was made to pass over the substrate. The UV-Vis spectrum was obtained after the 5 minutes of reaction time. Figure 5.22 shows the changes in the UV-Vis spectra obtained from the CdS nanoparticle samples with varying concentration of CdCl₂ concentration. It was observed that the absorption peak was at ~465 nm, which can be attributed to CdS nanoparticles [115]. By increasing the CdCl₂ concentration from 0.8 mM to 1 M, several observations could be made. The spectra for 0.8 mM, 2.5 mM and 7 mM showed an unchanged absorption peak at ~465 nm. It was also observed that the UV-Vis spectrum appeared to shift (figure 5.22 inset) from ~465 nm to ~520 nm for 1 M CdCl₂, due to the increase in the size of the CdS nanoparticles as the concentration of CdCl₂ increased.
Figure 5.22. UV-Vis spectra of CdS nanoparticle on cellulose with increasing concentration of CdCl\textsubscript{2}. The absorption peak was observed at \~465\,nm, which appeared to red shift \~520\,nm for 1M CdCl\textsubscript{2} (inset).

A similar experiment, as described above, was performed for ZnS nanoparticle synthesis. Here, it was observed that the absorption peak remains unchanged at \~310\,nm with increase in concentration of the salt solution, ZnCl\textsubscript{2} in this case. The absorption peak for 1M ZnCl\textsubscript{2} solution was also at \~310\,nm, indicating that the particle size remained more or less similar to all other concentrations. This observation could be due to the highly acidic solution of ZnCl\textsubscript{2} (pH = 2). An acidic medium will lead to the following reaction mechanism:

\[ \text{H}_2\text{S} + \text{H}_2\text{O} = \text{S}^{2-} + \text{HS}^- + \text{H}^+ + \text{OH}^- \] ....................................................... (1)

\[ \text{S}^{2-} + \text{H}^+ = \text{HS}^- \] ................................................................. (2)

In a basic medium the following reaction becomes valid:

\[ \text{HS}^- + \text{OH}^- = \text{S}^{2-} + \text{H}_2\text{O} \] ....................................................... (3)
Thus, in an acidic medium there are less S\(^{2-}\) ions available to react with Zn\(^{2+}\) and therefore less ZnS formed even as the ZnCl\(_2\) concentration was increased to 1M. Figure 5.23 shows UV-Vis spectra of ZnS nanoparticles on the cellulose template.

![UV-Vis spectrum](image)

Figure 5.23. UV-Vis spectra of ZnS nanoparticles on cellulose with increasing concentration of ZnCl\(_2\). The absorption peak was observed at ~310 nm. The inset shows another UV-Vis spectrum with an absorption peak at ~310 nm for 1M ZnCl\(_2\) solution.

From the UV-Vis results it was evident that the particle size could be varied by varying the concentration of the salt solution. However, it was also important to note that the pH of the salt solution could have an influence on the particle size (Figure 5.23). Therefore the next step in the synthesis of varying particle size was to obtain a control over the particle size by changing the pH of the salt solution. The reaction time was kept constant at 5 min, and the pH of the salt solution was varied between 4 and 7. Figure 5.24 shows two TEM images of CdS nanoparticles on cellulose. It was observed that the particle size increased with increase in the pH of the salt solution. It was also observed that the coverage of the cellulose template improved with an increase in the pH of the salt solution.
Figure 5.24. TEM images of CdS nanoparticle samples with varying pH of the CdCl\textsubscript{2} solution (a) \( \text{pH} = 4.0 \) and (b) \( \text{pH} = 7.0 \)

Similarly, experiments were carried out for PbS and ZnS nanoparticle samples. Figure 5.25 and 5.26 show two TEM images of ZnS and PbS nanoparticle samples indicating similar trends. The particles increase in size with increase in pH of the salt solution. The coverage of the template also appears to improve with increase in pH.

Figure 5.25. TEM images of ZnS nanoparticle samples with varying pH of the ZnCl\textsubscript{2} solution (a) \( \text{pH} = 4.0 \) and (b) \( \text{pH} = 7.0 \)
The next step in the particle size variation experiment was to perform a similar set of experiment with constant pH and with varying reaction time (H₂S exposure time). The synthesis procedure was followed as described above with a variation of the reaction time from 2 to 10 minutes. The pH of the salt solution, CdCl₂ in this case, was at a value of 6.5. Figure 5.27 shows three TEM images of CdS nanoparticle samples synthesized with varying reaction time. It was observed that the particle size increased with increase in the reaction time. Along with increase in the size of the nanoparticles, the coverage of the cellulose appeared to have improved. Figures 5.27 (a), when compared to Figures 5.27 (b) and (c) clearly showed fewer gaps between CdS nanoparticles. The inset in figure 5.27c shows the highlighted region. Here the morphology of the CdS nanoparticles can be clearly seen.
Figure 5.27. TEM images of CdS nanoparticles on the cellulose template with varying reducing time, (a) 2 min, (b) 5 min, (c) 10 min.

Similar results were observed for PbS nanoparticle samples on the cellulose template. There was an increase in the particle size with increase in the reaction time from 2 to 10 min. Figure 5.28 shows three TEM images of PbS nanoparticles on cellulose. The inset shows the highlighted region, which clearly shows the morphology of the PbS nanoparticles. Similar trends were observed for ZnS nanoparticle samples, (shown in figure 5.29).
Figure 5.28. TEM images of PbS nanoparticles on cellulose with varying reducing time, (a) 2 min, (b) 5 min, (c) 10 min.
Figure 5.29. TEM images of ZnS nanoparticles on cellulose with varying reducing time, (a) 2 min, (b) 5 min, (c) 10 min.

Although the particle size and the coverage of the template increased with increase in concentration of salt solution, pH of the salt solution and reaction time, there were certain regions along the template that still remained exposed. In order to further improve the coverage of cellulose, another set of experiments were carried out. In this experiment the temperature of the salt solution was increased from room temperature to
85°C. The synthesis process was similar to that described above with the exception of using the salt solution that was incubated for 30 min at 85°C. Figure 5.30 shows a TEM image of CdS nanowire on cellulose obtained after incubating the salt solution at 85°C. Here, the nanoparticles cover the entire template and the nanostructure appears like a wire. Similar results were obtained for the PbS and ZnS samples, shown in figure 5.31 and 5.32 respectively.

Figure 5.30. TEM image of CdS nanowire on the cellulose template, obtained after incubating the salt solution at 85°C for 30 min.
Figure 5.31. TEM image of PbS nanowire on the cellulose template, obtained after incubating the salt solution at 85ºC for 30 min.
Figure 5.32. TEM image of ZnS nanowire on the cellulose template, obtained after incubating the salt solution at 85ºC for 30 min.

5.6 Conclusion

In conclusion, metallic (Ag, Au, Cu and Pt) and semiconducting nanoparticles (CdS, PbS and ZnS) were successfully synthesized on the cellulose template. The surfactant CTAB helped in the synthesis of metallic and semiconducting nanoparticles on cellulose. The nanoparticles were characterized by UV-Vis, EELS and HRTEM. The EELS spectra confirmed the formation of metallic and semiconducting nanoparticles on cellulose. The EELS data distinctively showed Ag, Cd, Pb, Zn and S edges. The HRTEM images indicated a crystalline nature of the synthesized nanoparticles. The nanocrystals were in a size range of 2 – 6 nm. The nanoparticle size was varied by varying the process
variables, such as concentration of the salt solution, pH of the salt solution and the reaction time. The higher incubation temperature of the salt solution improved the coverage of the template, in case of semiconducting nanoparticles.

From the above results it can be concluded that cellulose can be used as a template for the fabrication of metallic and semiconducting nanoparticles. The cellulose fiber is inexpensive and has several other advantages as mentioned above and can be a viable alternative to the expensive biotemplates like DNA or viruses.
6.1 Introduction

A particular area in the field of biosensing has gained immense attention due to the need for the detection of highly toxic organophosphorous compounds that are commonly used as pesticides and chemical warfare agents [116]. The presence of these pesticides in our body can cause respiratory paralysis and even death [117]. The detection of these pesticides becomes extremely important with regards to public health, environment and food safety [118, 119]. There have been several successful attempts to detect pesticides by immobilizing enzymes on nanostructures. The nanostructures that have been used as platforms for the purpose of biosensing were gold nanoparticles [120], carbon nanotubes [121], zirconia nanoparticles [122], gold-polypyrrole network [123] etc. All the mentioned nanostructures were synthesized by relatively complicated synthesis techniques. These synthesis routes either required a precise control of temperature or time. The bottom up technique used in the synthesis of silica (SiO₂) and titania (TiO₂) nanowires for a biosensing application was not explored. The alpha synuclein template was used in this study due to the special properties of this template
such as; it has ideal dimensions for nanowire synthesis, it has a net negative charge on the surface and it can be synthesized in the laboratory with relative ease [55]. The bottom up synthesis method provides mild experimental conditions for the fabrication of SiO$_2$ and TiO$_2$ nanowires, to be used as platforms for immobilizing enzymes. The porous structure of silica nanowires facilitates efficient immobilization of the enzymes for biosensing purposes. The synthesized nanowires provide a larger surface area as compared to the sol-gel membranes used by other researchers [123, 124], which indicates promise of better results in enzyme immobilization. This chapter presents the detailed synthesis of SiO$_2$ and TiO$_2$ nanowires on an alpha synuclein template and the immobilization of acetylcholinesterase (AChE) as a model enzyme to show the potential of this platform for biosensing applications.

6.2 Fabrication of SiO$_2$ and TiO$_2$ Nanowires on Alpha Synuclein Template

The following paragraphs describe the synthesis and characterization of SiO$_2$ and TiO$_2$ nanowires on the alpha synuclein template.

The synthesis of SiO$_2$ and TiO$_2$ nanowires was carried out by using tetraethylorthosilicate (TEOS) as the Si precursor and tetrabutyltitanate (TBT) as the titanium precursor. The other chemicals used in the synthesis procedure were ethanol, acetylacetone and methanol. For SiO$_2$ nanowire fabrication, the alpha synuclein fibrils suspended in the phosphate buffer were taken in an Eppendorf tubes. To this, a mixture of ethanol and water (50:50 wt %) was added and then TEOS was added. After each addition the solution was thoroughly mixed. This solution mixture was then incubated
overnight. The solution is then centrifuged and washed with methanol to remove any unreacted TEOS remaining in the sample. The final sample contained SiO₂ nanowires suspended in methanol. For TiO₂ nanowires, the alpha synuclein fibrils in phosphate buffer were taken in an Eppendorff. To this, a mixture of TBT and acetylacetone (v/v = 1:1) in ethanol was added. The solution mixture was incubated overnight. It was later centrifuged and washed with methanol. Figure 6.1 shows a schematic representation of the fabrication process.

![Schematic Representation](image)

Figure 6.1. Schematic representation of the fabrication process for the synthesis of SiO₂ and TiO₂ nanowires

6.3 Results and Discussion

The morphology and average diameter of the nanowires was obtained after analyzing the FESEM images of SiO₂ and TiO₂ nanowires. Figures 6.2a and 6.2b show FESEM images of SiO₂ and TiO₂ nanowires respectively. The average diameter for these samples was approximately 25 nm. The nanowire diameter appears uniform along the length. The images also indicate that the morphology of the alpha synuclein template was preserved after the nanowire formation.
Figure 6.2. FESEM images of SiO$_2$ (a) and TiO$_2$ (b) nanowires. The average diameter was measured to be 25 nm.

Energy dispersive spectrum (EDS) spectrum was obtained from a SiO$_2$ nanowire sample to determine the elemental presence of Si and O on the substrate. Figure 6.3 shows a FESEM image (a) of a SiO$_2$ nanowire and the EDS spectrum (b) was taken from the region indicated in the image (a). The EDS spectrum distinctively shows the Si and O peaks at 1.75 and 0.5 keV respectively. The spectrum also shows the carbon and copper peaks which come from the copper TEM grid and carbon substrate of the TEM grid. From the EDS spectrum the presence of Si and O, on the substrate, was confirmed.
Similar results were obtained from the TiO$_2$ nanowire sample, shown in figure 6.4. Here the sample appears to have an oxygen rich spectrum. This could be due to the Si substrate used for this sample. The Ti peak appears at ~0.5, 4.5 and 5 eV.

Figure 6.3. FESEM of a SiO$_2$ nanowire (a). An EDS spectrum (b) of the SiO$_2$ nanowire sample from the highlighted region in (a).
Figure 6.4. FESEM of a TiO$_2$ nanowire (a). An EDS spectrum (b) of the TiO$_2$ nanowire sample from the highlighted region in (a).

In addition to the EDS spectrum, EELS spectra were obtained from the SiO$_2$ and TiO$_2$ nanowires to confirm the formation of SiO$_2$ and TiO$_2$ on the alpha synuclein template. Figure 6.5 shows a TEM image (a) of a SiO$_2$ nanowire and the inset shows the
highlighted region, where the EELS spectrum was obtained. The figure 6.5 b shows the EELS spectrum. The spectrum very clearly shows the Si L\textsubscript{2,3} major edge at 99 eV and the Si L\textsubscript{1} minor edge at 149 eV. The spectrum also shows the carbon edge at ~300 eV. The nature of the spectrum matches well with a reference SiO\textsubscript{2} spectrum. Thus the EELS spectrum confirms the formation of SiO\textsubscript{2} on the alpha synuclein template.
Figure 6.5. A TEM image of SiO$_2$ nanowire (a). An EELS spectrum of SiO$_2$ nanowire (b) indicating the formation of SiO$_2$ nanowire on alpha synuclein template.

Similarly, an EELS spectrum was obtained from the TiO$_2$ nanowire sample to confirm the formation of TiO$_2$ on the alpha synuclein template. Figure 6.6 shows a TEM image (a) of a TiO$_2$ nanowire and figure 6.6b shows the EELS spectrum obtained from
the region shown in figure 6.6a. The EELS spectrum distinctively shows the Ti L\textsubscript{2} and L\textsubscript{3} major edges at 456 and 462 eV respectively. The Ti L\textsubscript{1} minor edge at 564 eV is observed. The oxygen O – K edge at 532 can also be seen. From the EELS spectra the formation of TiO\textsubscript{2} on the alpha synuclein template was confirmed. Moreover, the nature of the EELS spectrum matches well with the reference TiO\textsubscript{2} spectrum, which further confirms the formation of TiO\textsubscript{2}.
Figure 6.6. A TEM image of TiO$_2$ nanowire (a). An EELS spectrum obtained from (a) indicating the formation of TiO$_2$ on alpha synuclein template.

The synthesized nanowires could have applicability in various fields ranging from electronics to biosensing. Here the surface morphology of SiO$_2$ nanowires was exploited
for immobilizing an enzyme, for a biosensing application. This demonstrates the use of biotemplated nanowires as biosensing platforms. In order to use the surface of these synthesized nanowires and to obtain optimum biosensing response, the tuning of the surface area is necessary. The surface area of SiO$_2$ and TiO$_2$ nanowires can be varied by varying the process variables. The diameter of these nanowires could be varied from 25 nm – 100 nm. In case of SiO$_2$ nanowires the diameter was varied by varying the amount of SiO$_2$ precursor (TEOS). Figure 6.7 shows three TEM images of SiO$_2$ nanowires with varying diameter, from 20 – 100 nm, for 10 (a), 50 (b) and 200 ul (c) of TEOS respectively. From the TEM images it was observed that the morphology of the alpha synuclein template was maintained after the synthesis of SiO$_2$ nanowires with varying diameter. However, with the increase in SiO$_2$ precursor there was slight agglomeration of SiO$_2$ along the length of the nanowire.
Similarly, the diameter of TiO$_2$ nanowires could be varied by varying the precursors used in the synthesis process. Here, in this case the diameter could be varied by varying the amount of TBT and acetylacetone independently. The diameter of the TiO$_2$ nanowires could be varied between 20 – 100 nm. The figure 6.8 shows TiO$_2$ nanowires synthesized by varying the amounts of TBT from (a) 400 ul, (b) 1 ml, (c) 2 ml to (d) 4ml. The diameter of the nanowires increased with increase in the precursor volume. It was also observed that the morphology of the alpha synuclein template was maintained up to 2 ml TBT. Beyond 2 ml TBT the nanowire appeared to have agglomerates along their lengths, thus deviating from the morphology of the template.
This deviation was attributed to the increase in the precursor volume. Moreover, the TiO$_2$ nanowires appeared to have a greater contrast with increase in TBT. This was due to the increased amount of TiO$_2$ on the alpha synuclein template.

Figure 6.8. TEM images of TiO$_2$ nanowires with varying diameter, due to varying volumes of TBT (a) 400 ul, (b) 1 ml, (c) 2 ml and (d) 4 ml.

The diameter of the TiO$_2$ nanowires was also varied by varying the amount of acetylacetone. The precursor acetylacetone works as an anti gelation agent [125], which allows for slow gel formation of TiO$_2$. Thus the varying amounts of acetylacetone can be exploited to control the diameter of the TiO$_2$ nanowires. Figure 6.9 shows two TEM images of TiO$_2$ nanowires with varying amounts of acetylacetone. From the images it can
be clearly seen that the diameter varies with varying amounts of acetylacetone. The TiO₂ nanowire in figure 6.9a appears to have a larger diameter (~60 nm) than that of figure 6.9b (~40 nm). The acetylacetone volume for TiO₂ sample in figure 6.9a was 1 ml and for figure 6.9b was 5 ml.

Figure 6.9. TEM images of TiO₂ nanowires with varying diameter, due to varying volumes of acetylacetone (a) 1ml and (b) 5 ml.
6.4 Utilization of Biotemplated Silica Nanowires for Biosensing Applications

After the successful variation of the diameter in both SiO\textsubscript{2} and TiO\textsubscript{2} nanowires, the network like morphology of SiO\textsubscript{2} was exploited to demonstrate its use as a biosensor platform. The surface of the SiO\textsubscript{2} nanowires was used to trap or immobilize an enzyme, acetylcholinesterase (AChE) in this case. This enzyme was used to catalyze a hydrolysis reaction for acetylthiocholine chloride (ATCl) to produce an electroactive product of thiocholine. The activity of the enzyme was monitored by recording the electrical response after the addition of the substrate, which is ATCl in this case. The SiO\textsubscript{2} nanowires with entrapped enzymes were later incubated in a pesticide solution (paraoxon) for a known period of time. After the incubation the electrical response was recorded for the addition of substrate. The change in the electrical response was indicative of the presence of the pesticide. Figure 6.10a shows an FESEM image of SiO\textsubscript{2} nanowires on the electrode, which was used to obtain the electrochemical response. The figure 6.10b shows two current voltammetry (CV) curves recorded in the presence and absence of the substrate (ATCl). From these curves, it was evident that the hydrolysis reaction did not proceed in the absence of the substrate. However in the presence of the substrate an oxidation peak at 235 mV was recorded, indicating the oxidation of the reaction product, thiocholine. This oxidation potential of 235 mV was then applied to the electrode to obtain a calibration curve.
Figure 6.10. (a) FESEM image of several SiO$_2$ nanowires on the electrode surface. (b) Current-voltammetry curves obtained in the presence and absence of the substrate.

A calibration curve was obtained to study the extent of substrate required to saturate the available enzyme on the biotemplated SiO$_2$ nanowire surface, in order to record a maximum electrical response. To obtain a calibration curve, a small volume of the substrate was added and the electrical response was recorded. The addition was carried out in small steps until the response reached a maximum value. The electrical
response was observed to remain constant with any further addition of the substrate. The substrate volume was found to be 30 ul. Figure 6.11 shows the calibration curve, which clearly indicates that the electrical response saturates after a particular amount of substrate.

![Calibration Curve](image)

Figure 6.11. Calibration curve recorded to determine the amount of substrate required to saturate the available enzyme.

This volume of substrate (30 ul) was then used to study the electrical response of the enzyme before and after its exposure to the pesticide. The initial current response was obtained after the addition of 30 ul of substrate to the electrode, where the biotemplated SiO\textsubscript{2} nanowires and the entrapped enzymes were adsorbed. The initial current was found to be 0.4373 \textmu A. The electrode was then incubated in the pesticide solution for 10 min, and the final current response was recorded. The final current was found to be 0.0179 \textmu A. The decrease in the response indicates the presence of the pesticide. Thus the fabricated device, using SiO\textsubscript{2} nanowires as the biosensor platform can be used to detect the presence
of pesticides. Figure 6.12 shows the electrical response before and after the electrode was exposed to the pesticide.

![Graph showing electrical response before and after pesticide exposure](image)

Figure 6.12. Electrical response obtained before (a) and after (b) the electrode was exposed to the pesticide. The decrease in response indicates the presence of the pesticide.

From these results it was evident that the biotemplated SiO$_2$ nanowire morphology could be used as a platform for biosensing applications.
6.5 Conclusion

In summary, SiO$_2$ and TiO$_2$ nanowires were successfully synthesized on the alpha synuclein template. The nanowires were characterized by EDS and EELS. The EDS results indicate the presence of silicon, titanium and oxygen on the substrate. The EELS spectra confirm the formation of SiO$_2$ and TiO$_2$ on the alpha synuclein template. The diameter of the nanowires was varied by varying the process variables, such as the amount of precursor (TEOS, in case of SiO$_2$ nanowires and TBT, in case of TiO$_2$ nanowires) and the amount of acetylacteone, in case of TiO$_2$ nanowires. The surface of the silica nanowires was exploited for the immobilization of an enzyme to obtain an electrochemical signal for a biosensing application.

From the above results it can be concluded that the alpha synuclein template can be used in the fabrication of SiO$_2$ and TiO$_2$ nanowires for a biosensing application. The advantages of the bottom up technique can be utilized for an easy fabrication of biosensing platforms using mild experimental conditions. These biotemplated SiO$_2$ and TiO$_2$ nanowires provide a larger surface area when compared to other gel like networks or thin films used for similar purposes.
CONCLUSIONS

From the results obtained, it could be further concluded that amyloid like fibers could be used as biotemplates for the fabrication of 1D structures. The simple, inexpensive and mild experimental conditions make the synthesis process advantageous over other conventional synthesis routes. The control of the lateral dimensions of the nanowires helps to tune their properties. Moreover, the nature of the experimental conditions, the surface charge on the template, controlled reaction time and room temperature synthesis, helps to synthesize extremely small dimensions of nanocrystals (~2 nm) on the alpha synuclein template. Due to these small nanocrystals the synthesized structures could exhibit improved properties when compared with their bulk counterparts.

The use of a cellulose template is advantageous because it is inexpensive and abundantly available. It is also has dimensions similar to any other model template and has larger lengths when compared to that of the alpha synuclein protein template. The synthesized nanocrystals, on the cellulose template, have very small dimensions (~2 nm). The small size of the nanocrystals could provide improved properties. Due to these advantages the cellulose template can be a good alternative to the other relatively expensive templates.

The utilization of a biotemplate in the fabrication of SiO$_2$ and TiO$_2$ nanowires for a biosensing application is a unique approach to obtain a platform for immobilizing enzymes. The simple, inexpensive and mild synthesis route is advantageous over other
complicated methods, for the fabrication of such platforms. These biotemplated nanowires give satisfactory biosensing results. Moreover, if proper functionalization is carried out the synthesized nanowires could be patterned on the biosensing electrode for optimum response.
LIST OF REFERENCES


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VITA

Sonal Padalkar was born in the city of Pune, India. She completed her Bachelor’s degree in Metallurgical Engineering in 2001 from the Government College of Engineering, Pune at Pune University. She later completed her Master’s in Metallurgical Engineering from Pune University with a major in Physical Metallurgy. In 2005, she was admitted in the School of Materials Engineering at Purdue University to pursue a PhD degree under the guidance of Prof. Lia Stanciu.