

THE DEVELOPMENT OF FIBER OPTIC BIOSENSOR FOR THE DETECTION OF PROTEIN AGGREGATES

by

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**THE DEVELOPMENT OF FIBER OPTIC BIOSENSOR FOR
THE DETECTION OF PROTEIN AGGREGATES**

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ACADEMIC ETHICS AND INTEGRITY STATEMENT

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ABSTRACT

THE DEVELOPMENT OF FIBER OPTIC BIOSENSOR FOR THE DETECTION OF PROTEIN AGGREGATES

α -synuclein oligomers play a crucial role in the early diagnosis of Parkinson's disease (PD). In this study, a mercaptoundecanoic acid (MUA)-capped gold nanorod (GNR)-coated and chitosan (CH)-immobilized fiber optic probe has shown considerable sensitivity of its detection. The proposed U-shaped fiber optic biosensor based on localized surface plasmon resonance (LSPR) was applied to detect α -syn oligomer (OA) biomarker. By analyzing OA concentrations, the biosensor achieved a limit of detection of (LOD) 11 pM within the concentration range of 10-100 pM and the sensitivity value was found as $502.69 \Delta\lambda/RIU$. Upon analysis of the CV% (coefficient of variation) and accuracy/recovery values, it is revealed that the sensor successfully fulfilled the criteria for success, displaying accuracy/recovery values within the range of 80%-120% and CV% values below 20%. This sensor presents significant advantages, including high sensitivity, specificity, and ability to detect very low concentrations of OA. In conclusion, the suggested U-shaped fiber optic biosensor has the potential to be valuable in the early detection of PD from a clinical perspective.

Keywords: Localized Surface Plasmon Resonance, Parkinson's Disease, α -synuclein Oligomers, Chitosan, Fiber Optic Biosensor, Gold Nanorod, Oligomer α -synuclein.

ÖZET

PROTEİN BİRİKİMLERİNİN TESPİTİ İÇİN FİBER OPTİK BİYOSENSÖR GELİŞTİRİLMESİ

α -sinüklein oligomerleri, Parkinson hastalığının (PH) erken teşhisi açısından kritik bir rol oynamaktadır. Bu çalışmada, mercapto undekanoik asit (MUA) ile kaplanmış altın nanorod (GNR) içeren ve kitosan (CH) ile immobilize edilmiş fiber optik bir prob, algılama hassasiyeti bakımından önemli bir performans sergilemiştir. Önerilen U şekilli fiber optik biyosensör, lokalize yüzey plazmon rezonansı (LSPR) tabanlı olarak tasarlanmış ve α -sinüklein oligomer (OA) biyobelirteci tespiti için kullanılmıştır. OA konsantrasyonları analiz edilerek sensör, 10-100 pM konsantrasyon aralığında 11 pM tespit sınırı (LOD) ve $502.69 \Delta\lambda/RIU$ hassasiyet değerine ulaşmıştır. Değişim katsayısı (CV%) ve doğruluk/geri kazanım değerlerinin incelenmesi sonucunda, sensörün başarı kriterlerini karşıladığı ve doğruluk/geri kazanım değerlerinin %80-120 aralığında, CV% değerlerinin ise %20'nin altında olduğu belirlenmiştir. Bu sensör, yüksek hassasiyet, özgüllük ve çok düşük OA konsantrasyonlarını algılama yeteneği gibi önemli avantajlar sunmaktadır. Sonuç olarak, önerilen U-şekilli fiber optik biyosensörün, klinik açıdan Parkinson hastalığının erken teşhisinde değerli bir araç olma potansiyeli taşıdığı görülmektedir.

Anahtar Sözcükler: Lokalize Yüzey Plazmon Rezonansı, Parkinson Hastalığı, Alfa-sinüklein Oligomerleri, Kitosan, Fiber Optik Biyosensör; Altın Nanorod, Alfa-sinüklein Oligomer.

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LIST OF SYMBOLS

μM	Micromolar
μs	Microsecond
mM	Millimolar
pM	Picomolar
C	Concentration
$^{\circ}\text{C}$	Degrees Celcius
ml	mililiter
mm	milimeter
mM	milimolar
g	gram
μm	micrometer
μL	microliter
nm	nanometer
s	Second
min	minute
V	Volume
Pj	depolarization factor
e	ellipticity
RIU	Refractive index units

LIST OF ABBREVIATIONS

FO	Fiber Optic
FOS	Fiber-optic sensors
Cext	Extinction cross-section
LSPR	Localized surface plasmon resonance
PD	Parkinson's Disease
ELISA	Enzyme-Linked ImmunoSorbent Assay
MUA	11-Mercaptoundecanoic acid
GNRs	Gold Nanorods
CH	Chitosan
OAs	α -Syn Oligomers
FTIR	Fourier transform infrared spectroscopy
RI	Refractive Index
ThT	Thioflavin T (ThT) Fluorescence
L-SPR	Longitudinal Surface Plasmon Resonance
T-SPR	Transverse Surface Plasmon Resonance
MNPs	Metallic Nanoparticles
LOD	Limit of Detection
SERS	Surface-Enhanced Raman Scattering
TPB	Transverse Plasmon Band
LPB	Longitudinal Plasmon Band (LPB)
AuNPs	Gold Nanoparticles
PoC	Point-of-Care
EDC	1-Ethyl-3-(3-Dimethylaminopropyl)Carbodiimide
NHS	N-hydroxysuccinimide
PBS	Phosphate-Buffered Saline
SEM	Scanning Electron Microscopy
APTES	(3-aminopropyl)triethoxysilane
PoC	point of care

SAM

self-assembled monolayer



1. INTRODUCTION

1.1 Motivation

Parkinson's disease (PD) is a progressive neurological disorder that affects millions globally, leading to severe motor symptoms like tremors, muscle stiffness, and slowness of movement [1]. Despite its widespread occurrence, diagnosing PD early and accurately remains a major challenge for clinicians. A key feature of PD is the abnormal buildup of the α -synuclein (α -syn) protein in the brain, which is thought to play a significant role in the disease's development [2]. Detecting α -syn, particularly its oligomeric forms, at the early stages of the disease is crucial because these forms are closely linked to the initial progression of PD and the resulting neuronal damage. Early detection could lead to timely interventions, potentially slowing disease progression and improving outcomes for patients [1, 2].

Currently available methods for detecting α -syn oligomers, like ELISA and immunoassays, come with several limitations [3]. These include high costs, long processing times, and difficulty in distinguishing between different harmful forms of α -syn, such as oligomers and fibrils. This research is driven by the need for a more efficient, sensitive, and cost-effective approach to detecting α -syn oligomers. Developing a reliable tool capable of detecting these oligomers at low concentrations could not only deepen our understanding of PD but also help improve clinical outcomes by facilitating earlier diagnosis. In addition, fiber optic biosensors have other main advantages, such as their small and portable package size, which improves their ease of integration into clinical environments [4]. They can respond rapidly with a small sample amount making them truly pragmatic for diagnostics in the real world. Optical fibers provide flexibility for the design of unique probes thus adaptation to a broad range of uses such as real-time monitoring and in situ biomarker monitoring.

U-shaped fiber optic probes with LSPR create the most advanced detection

technologies. Their broad geometrical shapes increase light-analyte interactions to enhance detection for analytes that exist in very low concentrations [5], such as the α -synuclein oligomers. In this way, such novel processes could have an extraordinary impact on the diagnostic design, namely, the detection of disease-related markers at nanomolar or even lower levels [5].

This research is motivated by the need to overcome the constraints existing in the current detection schemes, providing fresh and precise solutions through LSPR-based U-shaped fiber optic biosensors. Acting by designing a sensor to detect α -syn oligomers with high sensitivity and specificity, this work goes a long way in filling a critical gap in early detection of Parkinson's disease that is vital for enhanced patient outcomes and timely therapeutic interventions.

1.2 Objectives and Outline of the Thesis

The main goal of this research is to develop a highly sensitive and specific method for detecting α -synuclein (α -syn) oligomers, which play a crucial role in the early stages of Parkinson's disease (PD). This study aims to create and implement a new fiber optic biosensor based on localized surface plasmon resonance (LSPR) technology. This sensor is designed to overcome some of the limitations of current detection methods such as ELISA and immunoassays by offering higher sensitivity, lower costs, and faster response times. By focusing on the detection of α -syn oligomers at low concentrations, the research seeks to improve early diagnosis, allowing for timely interventions that could help slow down the progression of the disease. The specific objectives of this thesis are:

1. To design a U-shaped fiber optic biosensor using LSPR technology that is specifically optimized for detecting α -syn oligomers.
2. To develop and fine-tune the biosensor using gold nanorods (GNRs) functionalized with 11-Mercaptoundecanoic acid (MUA) and chitosan (CH), enhancing the

sensitivity and specificity of detecting α -syn oligomers.

3. To test the biosensor's ability to detect α -syn oligomers at very low concentrations, particularly in conditions that simulate the early stages of Parkinson's disease.
4. To compare the biosensor's performance with existing diagnostic methods, showcasing the advantages and potential for clinical application.

This thesis is structured into 5 main chapters, each addressing a specific aspect of the research. The first chapter provides an introduction to Parkinson's disease, focusing on the challenges of early diagnosis and the importance of detecting α -synuclein oligomers. It also introduces the potential advantages of using LSPR-based fiber optic biosensors for early detection, setting the stage for the study. The second chapter presents a comprehensive review of the current diagnostic methods for Parkinson's disease, particularly those targeting α -synuclein. In this chapter, the strengths and limitations of these existing techniques are discussed, along with an overview of LSPR theoretical and its applications in biosensing. In the third chapter, the materials and methods used to develop the LSPR-based fiber optic biosensor are described in detail. This includes the design and fabrication process of the fiber optic sensor probe, the functionalization of gold nanorods with MUA and chitosan, and the experimental procedures used to optimize and test the sensor's performance.

Chapter four presents the results of the study, including an analysis of the biosensor's sensitivity and specificity in detecting α -syn oligomers. The findings are compared with those of existing diagnostic methods, and the potential clinical applications of the sensor are explored.

Finally, the fifth chapter concludes the thesis by summarizing the key findings and discussing the limitations of the study. It also suggests directions for future research, particularly in terms of improving the biosensor's capabilities and expanding its use in diagnosing other neurodegenerative diseases.

2. BACKGROUND

2.1 α -syn and Parkinson's Disease

The human brain holds a significant quantity of α -syn which is a 140 amino acid protein primarily located in presynaptic synapses [6]–[8]. Parkinson's disease, associated with α -syn buildup, has an increased risk linked to α -syn gene locus triplication, leading to higher α -syn levels [6, 9, 10]. Moreover, specific α -syn variations, such as A53T and A30P, have been implicated in PD, [11, 12] hindering the breakdown of α -syn, leading to its accumulation [13]. Monomeric α -syn changes into oligomers when the equilibrium between α -syn production and clearance is disrupted (shown in Figure 2.1) [14]. Recent evidence emphasizes the crucial role of oligomeric α -syn, detectable in cerebrospinal fluid (CSF), blood, and saliva, in the onset of PD [15]–[19]. Studies have identified α -syn oligomers (OAs) in the blood and CSF of PD patients, hinting at their potential use as diagnostic biomarkers [20]–[23].

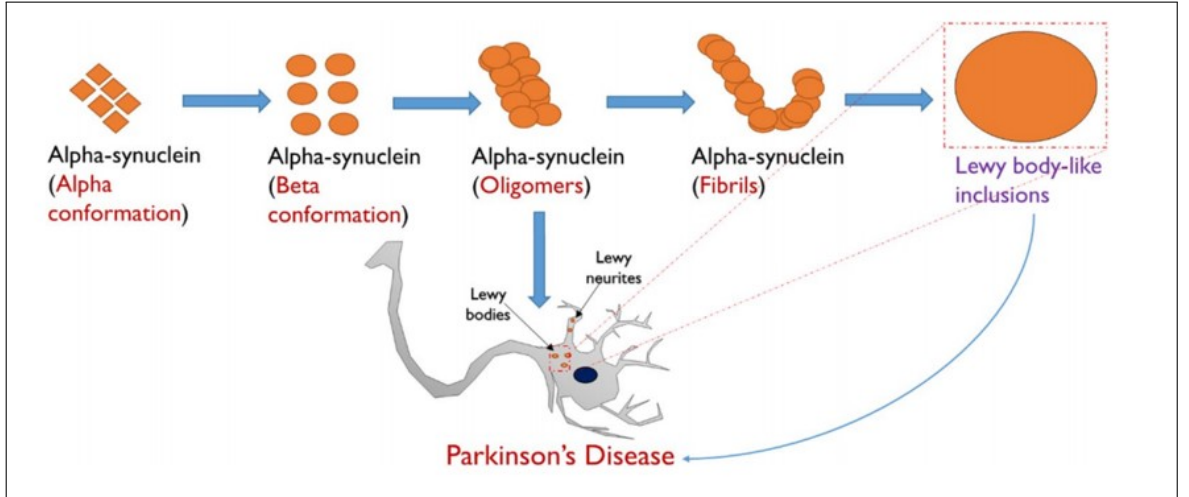


Figure 2.1 α -syn fibril aggregate formation from the monomeric form [24].

2.2 Challenges in Detecting α -Synuclein Oligomers

Traditional methods for detecting oligomer α -Synuclein include ELISA and single molecule immunoassays that utilize highly specific antibody pairs [25]–[28]. Yet, developing antibodies specific to pathogenic α -syn assemblies, including oligomers, remain challenging as current aggregate-specific antibodies cannot distinguish between oligomers and fibrils [29]. Other than immunoassays techniques such as Circular dichroic [30], Fourier transform infrared spectroscopy (FTIR) [31], Western blot [32], Magnetic resonance imaging (MRI) [33] and thioflavin T (ThT) fluorescence [34] are among the techniques used to detect OAs. However, these methods have limitations such as high cost, slow response time, and the need for bulky instruments. Hence, the oligomer-based α -syn protein detection technique stands out due to its simplicity, high sensitivity, and specificity. Nevertheless, longitudinal surface plasmon resonance (LSPR)-based fiber optic biosensors exhibit high precision, specificity, and sensitivity in protein detection [35]. Additionally, these biosensors offer rapid response times, portability, and suitability for on-site applications [36]. Therefore, LSPR-based fiber optic biosensors hold promise as effective alternatives for α -syn detection.

2.3 LSPR and Fiber Optic Biosensors

Fiber optic biosensors operate based on the coherent interaction between incoming photons and metal nanoparticles. Among the commonly used metal nanoparticles in LSPR applications are GNRs, distinguished by two absorption bands: the longitudinal surface plasmon resonance (L-SPR) and the transverse surface plasmon resonance (T-SPR). L-SPR and T-SPR represent electron oscillation along the longer and shorter axes of GNRs, respectively. The sensitivity of GNRs to changes in size and the surrounding medium's refractive index (RI) varies between L-SPR and T-SPR. TSPR demonstrates lower sensitivity to these alterations, while L-SPR exhibits a shift to longer wavelengths (redshift) in response to an increased aspect ratio of GNRs, displaying heightened sensitivity to RI changes [37]. The characteristics of LSPR heavily

rely on nanoparticle size, shape, dielectric properties, and the nature of the surrounding environment, impacting the electron charge density on the particle surface [38, 39]. LSPR is highly responsive to changes in the RI within the local environment. In numerous sensing methodologies, alterations in peak wavelength or absorbance in the absorption spectrum of MNPs are employed as indicators of LSPR sensor response. Another crucial factor influencing the effectiveness of LSPR-based fiber optic biosensors is the design of the FO probe. Studies have indicated that U-shaped FO probes can significantly enhance sensitivity, especially for biomarkers with low concentrations, like oligomeric α -syn, due to their heightened RI sensitivity [37].

2.4 Surface Functionalization Techniques and Design of Sensor Probe

11-Mercaptoundecanoic acid (MUA) is an organic compound which is used to form a self-assembled monolayer on the surface of GNR, thereby enhancing sensor performance [40]. This enhancement encompasses the improvement of target molecule binding to the sensor surface, an increase in detection sensitivity, and the establishment of stability in the sensor surface [38, 39]. MUA was selected for the surface functionalization of nanoparticles due to its non-toxic properties [41]. The interaction between MUA and GNRs is attributed to the affinity of thiol groups for the gold nanoparticles [42]. Consequently, MUA emerges as the most suitable molecule for establishing a stable surface within the scope of this study.

Chitosan is made by processing chitin to remove its acetyl groups ($-\text{COCH}_3$) (shown in Figure 2.2). This can be done using enzymes or by applying heat in an alkaline solution. Once more than half of these acetyl groups are removed, it transforms into chitosan. Chitosan (CH), a carbohydrate polymer, has emerged as a valuable transducer owing to its robust mechanical properties, strong adhesive behavior, biocompatibility, biodegradability, high availability, and sustainability [43]. As a polysaccharide, CH can interact with amyloid molecules through its glucosamine groups, positioning

it as a promising candidate for sensor applications [44, 45]. Chitosan features a side-amino group in its carbon chain, which has a pKa value of 6.5, thus becoming positively charged when the pH is lower than 6.5. This specificity makes it more interesting when compared with most other natural polysaccharides like alginate or guar gum, which mostly maintain neutral or negative charges. Its positive charge makes chitosan a versatile polymer moiety that can participate in several interactions with negatively charged molecules, including nucleic acids or nanomaterials, presenting potential applications in the production of immunoassay sensors [46].

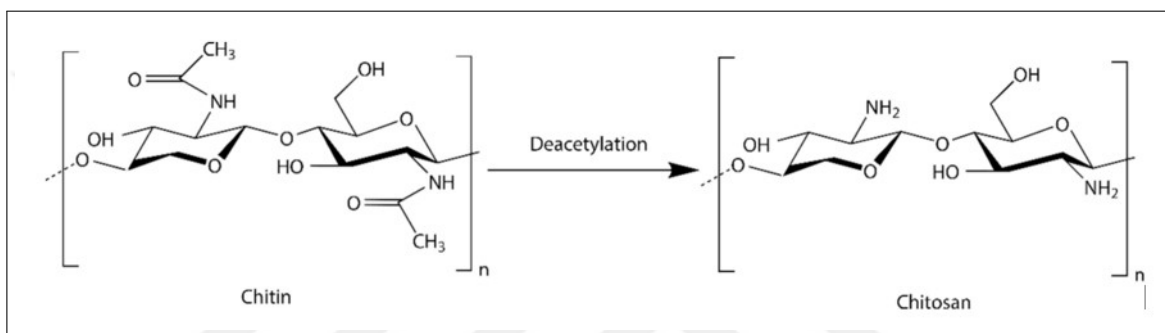


Figure 2.2 Chitosan is formed by deacetylation of chitin [47].

In this study, U-shaped LSPR based fiber optic biosensor probe was specifically tailored for detecting α -syn oligomers MUA-capped GNRs were immobilized onto the fiber-optic probe's core and bio-functionalized with CH to create the sensing region on the fiber Figure 2.3 and experimental set was shown in Figure 2.4. The outcomes of this study demonstrated that the biosensor developed using a CH-coated sensor probe could detect α -syn oligomers with a low LOD and high selectivity.

2.5 Introduction to Localize Surface Plasmon Resonance (LSPR)

The LSPR phenomenon arises mainly when incident photons trigger the resonant oscillation of conduction electrons in metallic nanoparticles [48, 49]. The LSPR corresponds to a resonance of the electromagnetic field inside a metallic nanostructure whose size is smaller than the wavelength of the excitation optical wave. This resonance is intrinsically coupled to an oscillation of charges that can be viewed as a global oscillation of the conduction electrons inside the metal. This oscillation of charges

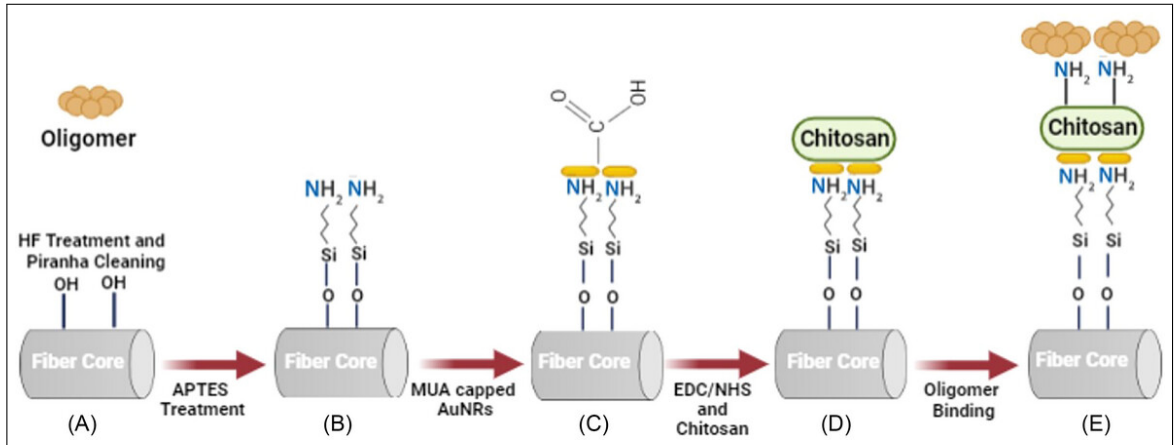


Figure 2.3 Chitosan-enhanced sensitivity of mercaptoundecanoic acid (MUA)-capped gold nanorod based localized surface plasmon resonance (LSPR) biosensor for detection of alpha-synuclein oligomer biomarker in Parkinson's disease.

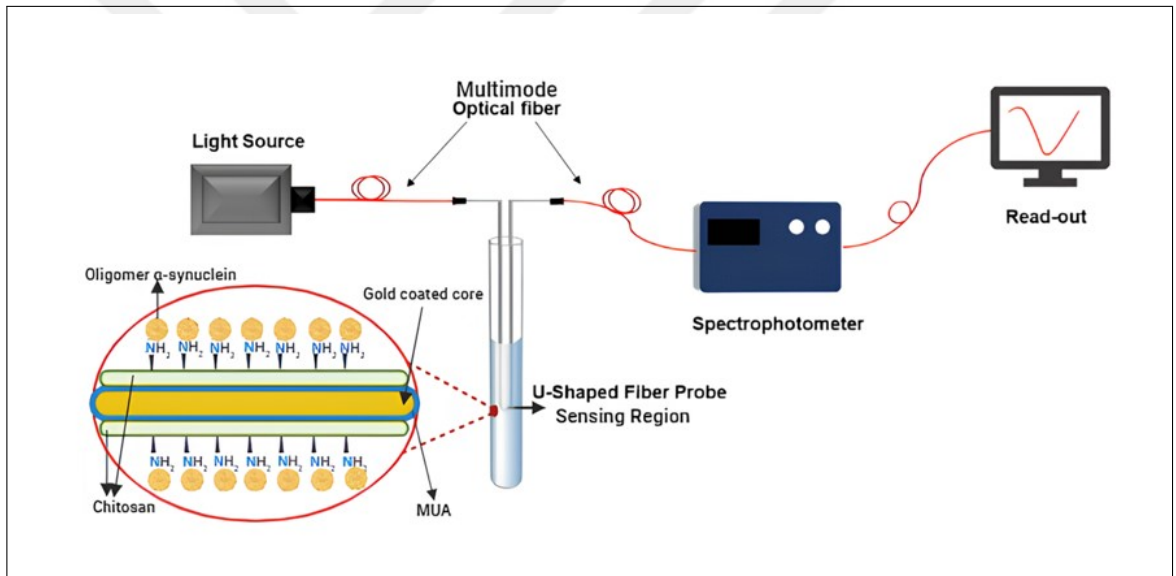


Figure 2.4 Schematic representation of the experimental setup for detecting oligomeric α -synuclein using a MUA capped GNRs coated U-shaped fiber optic probe. The setup consists of a light source, a multimode optical fiber with a gold-coated core and chitosan layer for capturing oligomeric α -synuclein, and a spectrophotometer for measuring the optical response. The sensing region of the U-shaped fiber is immersed in the sample, and the output signal is analyzed using a connected read-out system. The inset illustrates the functionalization of the fiber surface with MUA and chitosan for specific binding of α -synuclein oligomers.

would not occur in a bulk material with large dimensions, and its existence is linked to the constraints set by the boundaries of nanostructure [49]. LSPR is not a propagation but an oscillation of charges that eventually is coupled to an optical wave. The most striking characteristic of the LSPR is the strong optical absorption at the resonance

frequency, and when the latter is in the visible range, an assembly of nanoparticles acquire a very distinctive color. For example, gold nanoparticles appear red, and silver nanoparticles appear yellow. The Lycurgus cup (shown Figure 2.5) The most renowned example of the visual effect of LSPR is found in the Lycurgus cup, where nanoparticles made of gold and silver were employed for giving glass an intriguing red color. This cup is a carefully designed jewelry artwork dated to the fourth century and attributed to Roman glassmakers. This artwork is exhibited in the British Museum in London. On the cup, a mythological episode, the victory of the god Dionysus over Lycurgus, a king of the Thracians (circa 800 BC) is represented: One of Dionysus' maenads, Ambrosia, transformed into a vine by mother Earth, retains Lycurgus, while Dionysus instructs to kill him [49]. What is exceptional with the color of this glass is that it actually exhibits two colors: when observed in transparency, the cup appears red and when observed by diffusion, the color turns green [50]. Such an art piece is unique and was not well understood until recently [51]. The process used by the Roman glassmakers is still unknown, and it is unclear whether the Lycurgus cup was obtained by chance or was a controlled technology. The scientific explanations of the color of objects at that time were still very basic.

Michael Faraday wrote in 1857 that the color of the purple of Cassius was due to gold present in solution in a "finely divided metallic state" [52]. In the 19th century, many scientists tried to elucidate the origin of this purple coloration. Faraday's assumption was only demonstrated in 1905 by Richard Adolf Zsigmondy, who used a slit ultra-microscope, which is the ancestor of the dark-field microscope used for observing the light scattered by finely divided gold particles reduced by stannic acid. The complete story can be found in Carbert's article [53]. Gustav Mie, in 1908, confirmed these peculiar optical properties of small particles by providing a detailed analytical model of the absorption and scattering efficiency of metallic particles [54]. The Mie theory remains the reference for understanding the LSPR nowadays. But at that time, all the consequences had not been drawn, and the notion of the plasmon resonance had not been clarified. It was David Pines who first used the name "plasmon" in 1956 to describe the quantum of energy associated with the eigenfrequency of the collective oscillation of electrons in a metal [55]. This concept was applied to the SPP (surface

plasmon polariton) and the interest in plasmonic oscillations in nanostructures came into focus with the need to explain the incredible sensitivity of surface-enhanced Raman scattering (SERS) in the late 1970s, thanks in particular to the work of Richard Van Duyne [56].

Uwe Kreibig was the one who carried out the complete derivation of the localized plasmon resonance in nanoparticles in 1985 [57, 58]. Since then, the topic of localized plasmonic has been taken over by numerous scientists and applied to many fields. It benefited a lot from the huge progress in nano-optics and instrumentation.



Figure 2.5 The lycurgus cup fabricated during the Late Roman Empire, dated to the fourth century AD: The cup appears red when illuminated from inside and green when illuminated from outside [59].

2.5.1 Theoretical Approach to the LSPR in the Case of a Nanosphere

Understanding how a sphere scatters an incoming wave can provide valuable insights into everyday phenomena. Scattering occurs when light encounters particles that are close in size to the optical wavelength, explaining why fumes appear visible, clouds look white, and milk has a white, opaque quality. Metallic nanospheres, however, scatter light in unique ways. In addition to scattering, it's important to consider how much of the incoming wave's energy is absorbed and how much is transmitted. This is often described as the extinction efficiency of the nanosphere. Within the nanoparticle, the electric field can reach a strong resonance at a specific wavelength, resulting in a notable increase in optical absorption at that wavelength and a corresponding shift in

color [60].

The initial approach to understanding the scattering behavior of particles was introduced by Lord Rayleigh in 1871, specifically for cases where the particle diameter (D) is smaller than the wavelength of light (λ), denoted as $D \ll \lambda$. Under this condition, the electromagnetic field remains uniform across the entire nanosphere, a situation known as electrostatic approximation. In this scenario, the nanosphere acts like a dipole, with positive and negative surface charges symmetrically distributed around its center [61]. Rayleigh scattering, for example, serves as a foundational model for explaining how light interacts with ionized molecules in the atmosphere, which gives rise to the blue color of the sky. This approximation holds valid as long as the particle size remains very small, approximately $D < \lambda/100$ [62].

However, as the particle size approaches the wavelength of light ($D \approx \lambda$), a more precise framework is provided by Mie theory, developed by Gustav Mie in 1908 [63]. Mie theory offers an exact solution for the interaction between a plane wave and a nanosphere. The central concept of this theory is to represent the incoming plane wave in spherical coordinates centered on the nanosphere, enabling a detailed expansion in terms of multiple contributions such as dipolar, quadrupolar, and octupolar components (Figure 2.6). By applying appropriate boundary conditions for electromagnetic fields at the interface between the metallic sphere and its surroundings, the expansion coefficients can be accurately determined.

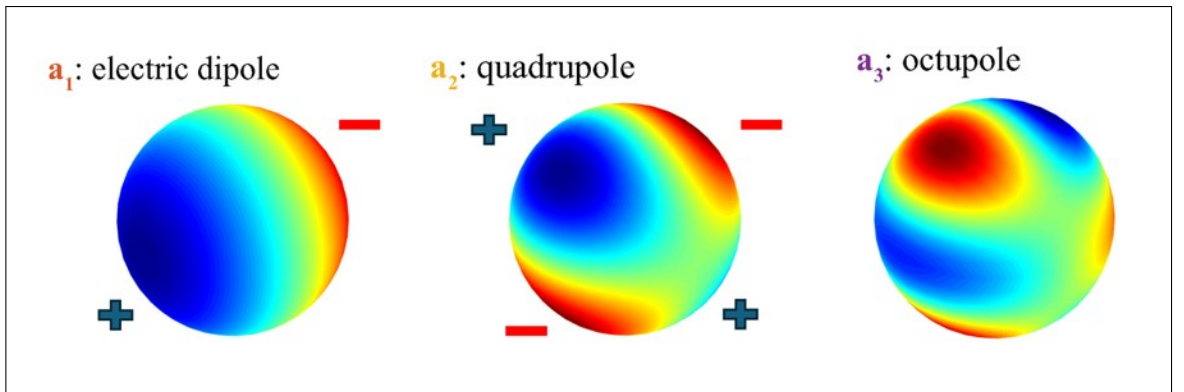


Figure 2.6 Charge oscillations of the nanosphere.

Figure 2.6 illustrates charge oscillations in a nanosphere placed in a quasi-uniform electric field. The dipolar contribution is represented by one positive and one negative pole, while the quadrupole contribution shows two positive and two negative poles. The octupolar contribution expands this pattern to four positive and four negative poles. These distributions of surface charges on the nanosphere, resulting from detailed calculations, showcase the first three contributions—dipolar, quadrupolar, and octupolar [64].

Together, these components create an overall charge distribution that oscillates over time. For small metallic particles (with diameters ($D < 20$ nm)), it is often sufficient to limit the multipole expansion to its first term, the dipolar contribution—known as the Rayleigh limit or the electrostatic approximation (also referred to as the quasistatic approximation) [65].

2.5.2 Theoretical Approach to the LSPR in the Case of a Nanorods

Gold nanorods shape and sizes spanning from a few to several hundred nanometers, stand out for their distinct optical characteristics, which differ significantly from those of spherical gold nanoparticles [66]. This difference is because of LSPR. The unique structure of nanorods causes electrons to polarize at different intensities depending on direction, as seen in Figure 2.7 [66, 67]. These nanorods support independent oscillations of electrons along both their width and their elongated axis. Along the shorter axis, electrons resonate within a wavelength range of about 510–530 nm, known as transverse surface plasmon resonance absorption (T-SPR). In contrast, electron vibrations along the longer axis led to light scattering that spans from visible to near-infrared regions, referred to as L-SPR. By adjusting the synthesis to control the aspect ratios of nanorods, it is possible to shift the longitudinal LSPR peaks from visible light to the near-infrared range. This longitudinal resonance, with its greater oscillator strength, becomes the main factor that defines the optical behavior of gold nanorods [68]. Gold nanorods' transverse surface plasmon resonance (T-SPR) tends to remain unaffected by changes in either their size or the RI of their environment. On

the other hand, their longitudinal surface plasmon resonance (L-SPR) shifts toward longer wavelengths (redshift) when the aspect ratio of the nanorods increases, making it highly sensitive to any shifts in the surrounding RI [69].

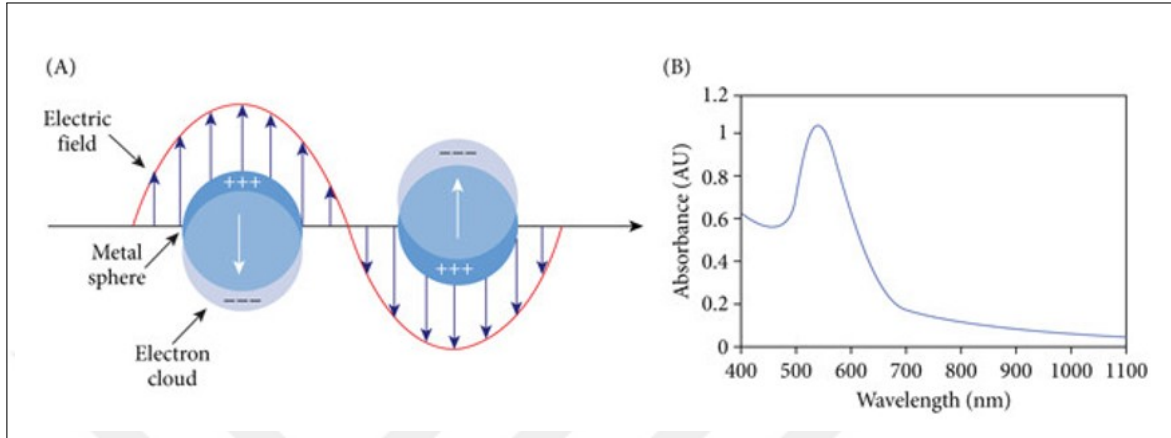


Figure 2.7 A) LSPR excitation for gold nanoparticle [61]. B) LSPR absorption spectrum for gold nanoparticles.

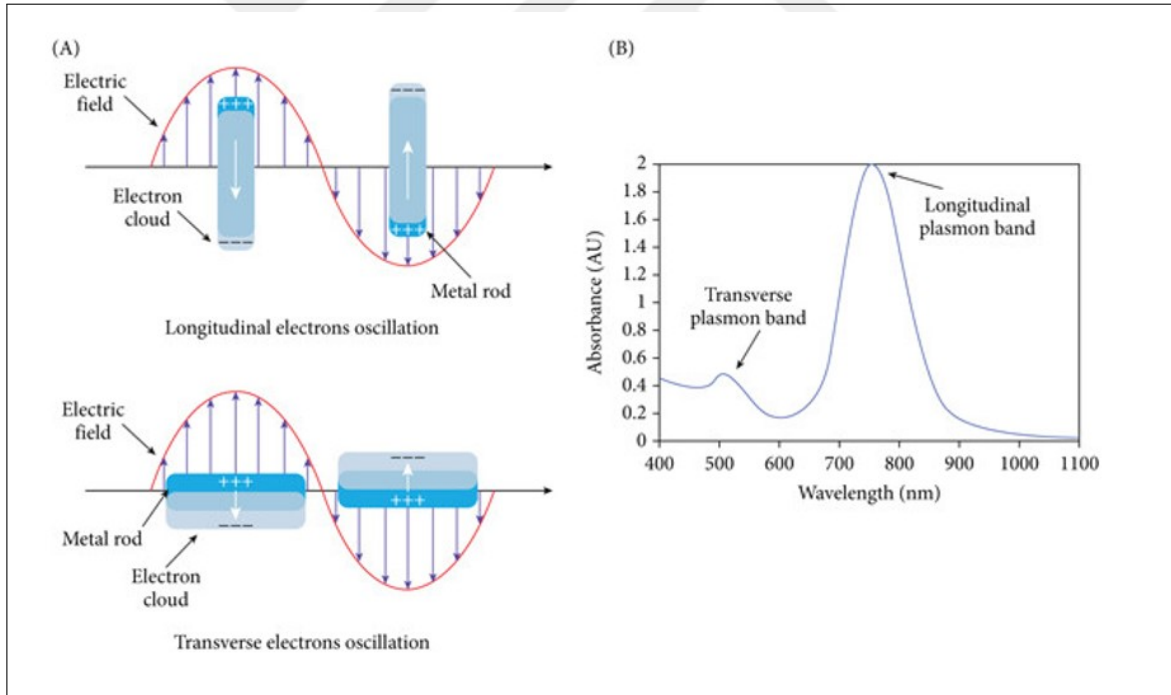


Figure 2.8 A) LSPR excitation for GNRs. B) LSPR absorption spectrum for GNRs [67].

Metallic nanorods, according to Gans [69], are predicted to exhibit a split in their surface plasmon modes into two distinct modes when considering small, ellipsoidal nanoparticles under the dipole approximation. This split occurs due to the specific curvature and shape of ellipsoidal particles. As a result, these nanorods are often modeled as ellipsoids to describe their optical characteristics [70, 71]. Thus, Gans'

theory is frequently applied to understand the optical behavior of metallic nanorods, allowing the extinction cross-section (C_{ext}) for these particles to be determined based on Gans' formula [72]–[74].

$$C_{ext} = \frac{2\pi V N \varepsilon_m^{3/2}}{3\lambda} \sum_j \frac{(1/P_j^2) \varepsilon_i}{(\varepsilon_r + ((1 - P_j)/P_j) \varepsilon_m)^2 + \varepsilon_i^2} \quad (2.1)$$

P_j and V are the depolarization factor and the volume of the particles, respectively. Polarization factor:

$$P_{\text{length}} = \frac{1 - e^2}{e^2} \left[\frac{1}{2e} \ln \left(\frac{1 + e}{1 - e} \right) - 1 \right] \quad (2.2)$$

$$P_{\text{width}} = \frac{1 - P_{\text{length}}}{2} \quad (2.3)$$

where e is the ellipticity, given by:

$$e^2 = 1 - \left(\frac{\text{length}}{\text{width}} \right)^{-2} \quad (2.4)$$

In this context, the aspect ratio of a nanorod is defined as the ratio of its length to its width. Localized surface plasmon resonance (LSPR) occurs when $\varepsilon_r = -((1 - P_j)/P_j)$. This equation suggests that even slight variations in the nanorod's aspect ratio can cause a significant shift in the plasmon band—an essential feature for sensing applications.

Any alterations in the surrounding medium of the nanoparticles lead to a shift

in the LSPR wavelength. This shift in LSPR wavelength, which occurs in response to changes in the refractive index, can be described by the following relationship [75, 76].

$$\Delta\lambda = m\Delta n \left[1 - \exp\left(\frac{-2d}{l_d}\right) \right] \quad (2.5)$$

In this context, Δn represents the refractive index changes, while m is the bulk refractive index response for nanoparticles, also referred to as the sensitivity (nm/RIU). Here, L_d is the decay length of the electromagnetic field (in nm). d stands for the thickness of the adsorbed layer (measured in nm). The Eq. 2.5 provides the foundation for LSPR wavelength-shift sensors, which use the LSPR shift to detect changes in the RI at the nanoparticle surface.

El-Sayed and co-workers simulated absorption spectra of GNRs at various aspect ratios in a constant dielectric medium using Gans theory in Figure 2.9. These two LSPR bands are T-SPR and L-SPR. With increased aspect ratio, GNRs show slightly blue shifted T-SPR, while I-SPR exhibits a considerable redshift. More important is the linear relationship of the peak wavelength of L-SPR with the aspect ratio, which perfectly fitted with experimental data. Figure 2.9B: The same study investigates how medium dielectric constants affect the absorption spectra of a GNR with fixed aspect ratio. The results indicated both T-SPR and I-SPR redshift upon increasing the dielectric constant. However, for LPB, both redshifts were significantly greater than for T-SPR, suggesting that the I-SPR is more sensitive to change in environmental dielectric properties. This implies that gold nanorods are quite sensitive in response to a change in the aspect ratio with respect to dielectric properties of the surrounding medium.

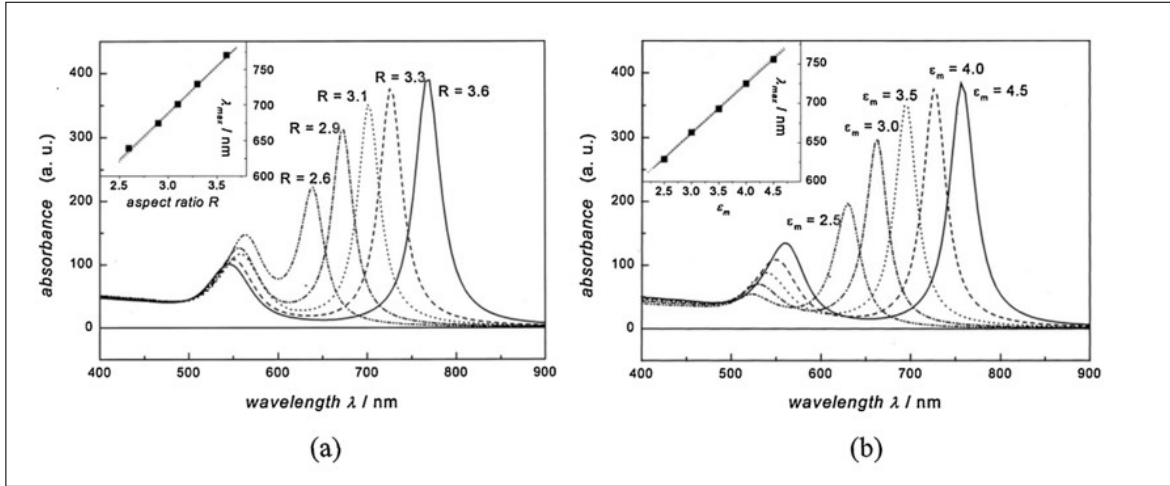


Figure 2.9 (a) Gold nanorods LSPR absorption spectra in a medium with constant dielectric constant with changing aspect ratios peak wavelength linearly grows. (b) GNRs with a different dielectric constant with a constant aspect ratios [67].

2.6 Gold Nanorods based Localized Surface Plasmon Biosensors

GNR-based LSPR biosensors are highly adaptable tools that allow for precise and targeted detection of biomolecular interactions across various platforms, such as Lab-on-a-chip sensors, Liquid-Phase Sensors, and Optical Fiber-based sensors. Each platform brings unique strengths to the table: Lab-on-a-chip sensors are well-suited for high-throughput analyses and easy integration into chip; and Liquid-Phase Sensors allow for real-time monitoring in liquid settings, making them particularly valuable for biochemical assays; optical fiber-based sensors offer flexibility and are ideal for remote sensing applications.

2.6.1 Lab-on-a chip Sensors

Lab-on-a-chip based LSPR sensors stand out for their enhanced consistency and ability to identify multiple targets at once, unlike colloidal nanoparticle systems. Typically, these sensors are designed using either evenly distributed nanoparticles across the substrate or structured periodic nanopatterns. GNRs and AuNPs have found extensive application in the development of LSPR sensors due to their unique optical

properties. Compared to colloidal particles, Lab-on-a-chip based platforms provide higher reproducibility and allow multiplex detection [77]. GNRs and AuNPs are often deposited on substrates like glass using techniques such as annealing, lithography, and chemical functionalization, enabling the fabrication of diverse nanostructures like nanopyramids, nanodisks, and nanoantennas [78]–[81]. These platforms offer enhanced signal-to-noise ratios and sensitivity, though their production can be complex and costly.

Recent developments in LSPR sensors based on flat substrates have increasingly focused on the integration of flexible nanostructures, driven by their practicality and versatility. One of the standout features of these sensors is their ability to perform real-time measurements and operate effectively in point-of-care (PoC) settings. To achieve this, researchers [82] in Figure 2.10 have incorporated a unique metal–insulator–metal (MIM) architecture into PDMS-based platforms.

A notable example of this innovation is a highly sensitive and stretchable LSPR sensor developed with a MIM-based structure. The fabrication process involved layering gold, silicon dioxide, and another gold layer onto an indium phosphide substrate, which was then embedded into PDMS. This combination created a flexible and biocompatible sensor material. By carefully adjusting the depth of the MIM disk within the PDMS layer, the researchers were able to enhance the device’s sensitivity and minimize interference from PDMS absorption. The resulting sensor demonstrated consistent performance even on bent or irregular surfaces, achieving a sensitivity of 3.5% with slight variations depending on the degree of curvature [82].

2.6.2 Liquid-Phase Sensors

Due to their presence in solutions, the use of nanoparticles offers substantial benefits for substrate fabrication as the nanoparticles can act as efficient LSPR substrates themselves without any need for additional steps for processing. Colloidal nanoparticles of different shapes (e.g., rods and spheres) are commonly used as LSPR

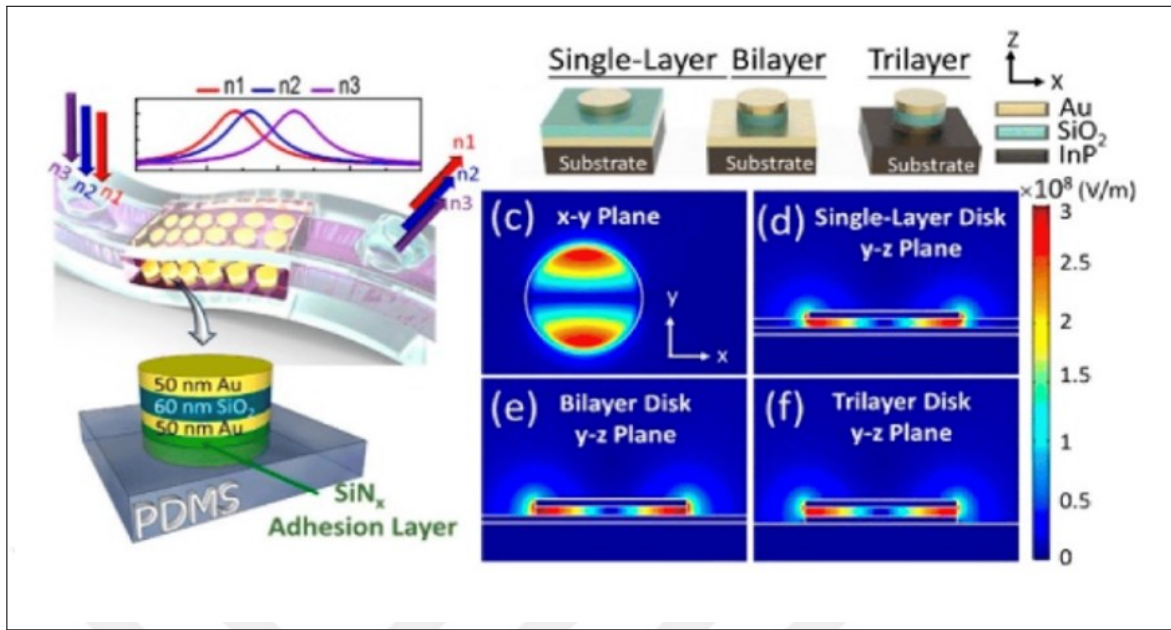


Figure 2.10 Metal-insulator-metal (MIM) Surfaces for detection of the cancer cells [82].

substrates and are often used as solution-based biosensors. Nanoparticles, nanorods, or nanowires aggregate or self-assemble into randomly distributed nanostructured colloids. These structures can produce “hot spots” at the poles of nanospheres or areas of high surface curvature, gaps, or junctions, resulting in enhanced sensitivity to refractive index changes [83]–[86].

This sensor allows in situ modification and even functionalization of GNPs being formed and also used for detection of the analytes directly in the GNP solution. In a recent study, gold core-satellite structures decorated with Hg^{2+} ion were used to design a GSH sensor ([87]; Figure 2.11). These structures were formed by crosslinking gold nanoparticles of two different diameters (50 nm for the core and 13 nm for the satellites) with DNA strands that had complementary sequences and served as base pairing linkers between the particles. As a result, the design utilized the strong affinity of GSH with the heavy metal ions such as Hg^{2+} , Cd^{2+} , and Pb^{2+} for GSH detection. Hg^{2+} was integrated into the system by inserting T- Hg^{2+} -T base pairing into the DNA duplex. When we added GSH to the T- Hg^{2+} -T composite solution, it bound more strongly to Hg^{2+} than T, so the T- Hg^{2+} -T interactions were disrupted, leading to disassembly of the structure and the accompanying significant blue shift in the LSPR peak. The system allowed the detection limit of $0.1 \mu\text{M}$ reached in 30 minutes.

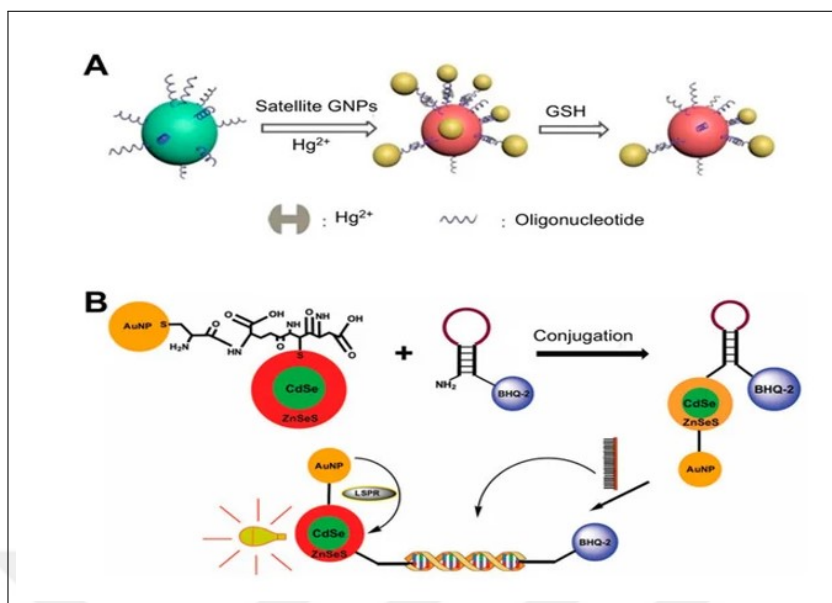


Figure 2.11 Solution phase-based nanoparticles: LSPR based on (A) By using Au core-satellite structures containing Hg^{2+} , detection of Glutathione (GSH) detection. (B) By using band-gap engineering, Detection of dengue virus with the usage of CdSe/ZnSeS core/alloyed shell quantum dots [87].

Solution-phase gold nanorod LSPR sensors utilize the unique optical properties of AuNRs, particularly their ability to detect changes in the surrounding refractive index by monitoring shifts in their longitudinal plasmon resonance band. In a study [88] these sensors were applied to detect the interaction between bovine serum albumin (BSA) and its antibody (anti-BSA), effectively capturing specific biomolecular binding events. The solution-phase configuration, where functionalized AuNRs are dispersed in suspension, achieved a sensitivity of 196 nm/RIU and a figure of merit (FOM) of 2.2 RIU^{-1} , demonstrating strong performance. Functionalization with mercaptoundecanoic acid (MUA) increased the surface area available for interaction, enabling real-time detection of these binding events in Figure 2.12. The solution-phase approach stood out for its simplicity, adaptability, and broad detection potential, making it a valuable tool for biosensing applications, particularly in antigen-antibody detection.

2.6.3 Optical Fiber-based Sensors

Fiber-optic sensors (FOS) are among the most studied research areas from the 1970s onwards [37]. They have some clear merits over the conventional sensor- biolog-

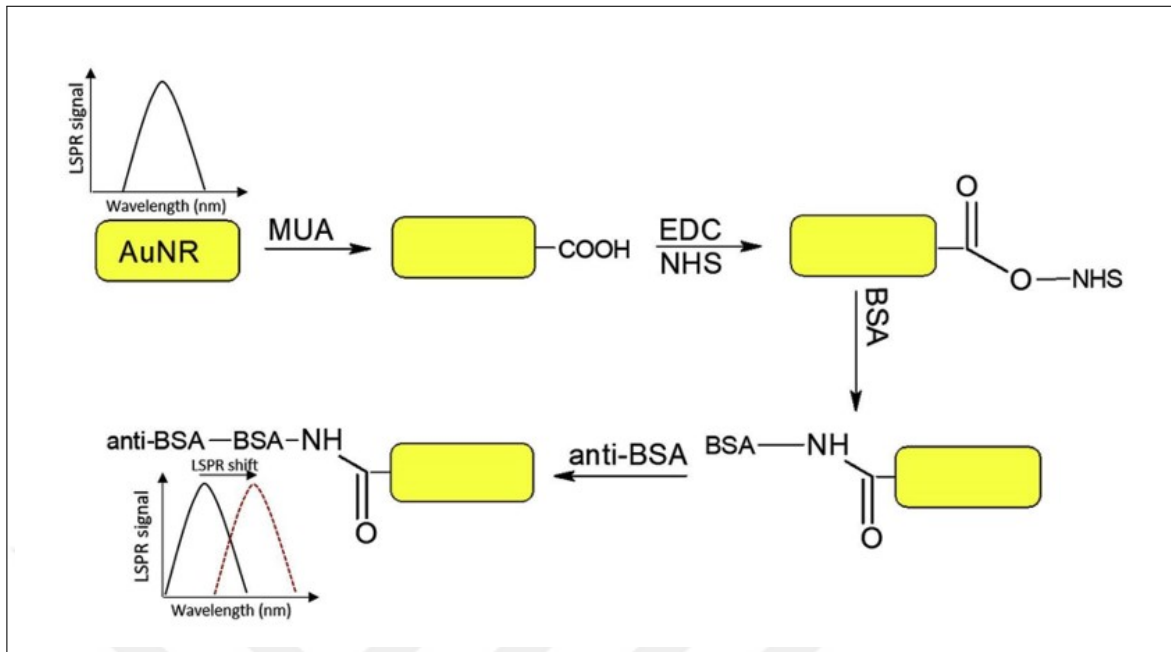


Figure 2.12 Functionalization of gold nanorods [88].

ical, electrochemical, and semiconductor-based systems. To highlight a few, FOS are small, light-weight, immune to electromagnetic interference, multiplexable, and remote sensing-ready. FOS's applications could be in the detection of temperature, pressure, strain, and acoustics, among others, besides being non-conductive [37].

Optical fibers: originally designed to transmit light well over long distances by total internal reflection with very little loss for the purpose of communication. They ultimately reduced the interaction of light with its “environment”, which is fundamental to the application of intrinsic fiber optic sensors systems. Usually, a section of the cladding is removed to facilitate access to that environment. The propagating light is prevented from directly refracting inward and a small amount, called the evanescent wave, is allowed to refract outward for interaction with the surrounding medium. Such interaction often results in the absorption or scattering of the evanescent wave, forming the basis of many sensing applications.

LSPR based fiber optic biosensors evaluate according to their geometry, fabrication, types and bio applications. Fiber-optic localized surface plasmon resonance (FOLSPR) sensors can be broadly categorized into two main types: straight transmissive and flat-headed reflective designs. Each of these has its own unique advantages

and can be adapted to meet specific sensing needs, depending on factors like the required sensitivity, the experimental setup, and the conditions in which the sensor will be used [89].

A variety of optical fibers can be incorporated into these designs, including single-mode fibers, multimode fibers, photonic crystal fibers and specialized options like microfibers and fiber Bragg gratings. Each fiber type brings something unique to the table—whether it's enhanced sensitivity, greater flexibility, or smoother integration with other systems. The shape of the sensor is just as important. Geometries like tapered, U-shaped, Ω -shaped, or D-shaped designs not only improve sensitivity but also allow the sensor to meet specific structural or assembly needs [90].

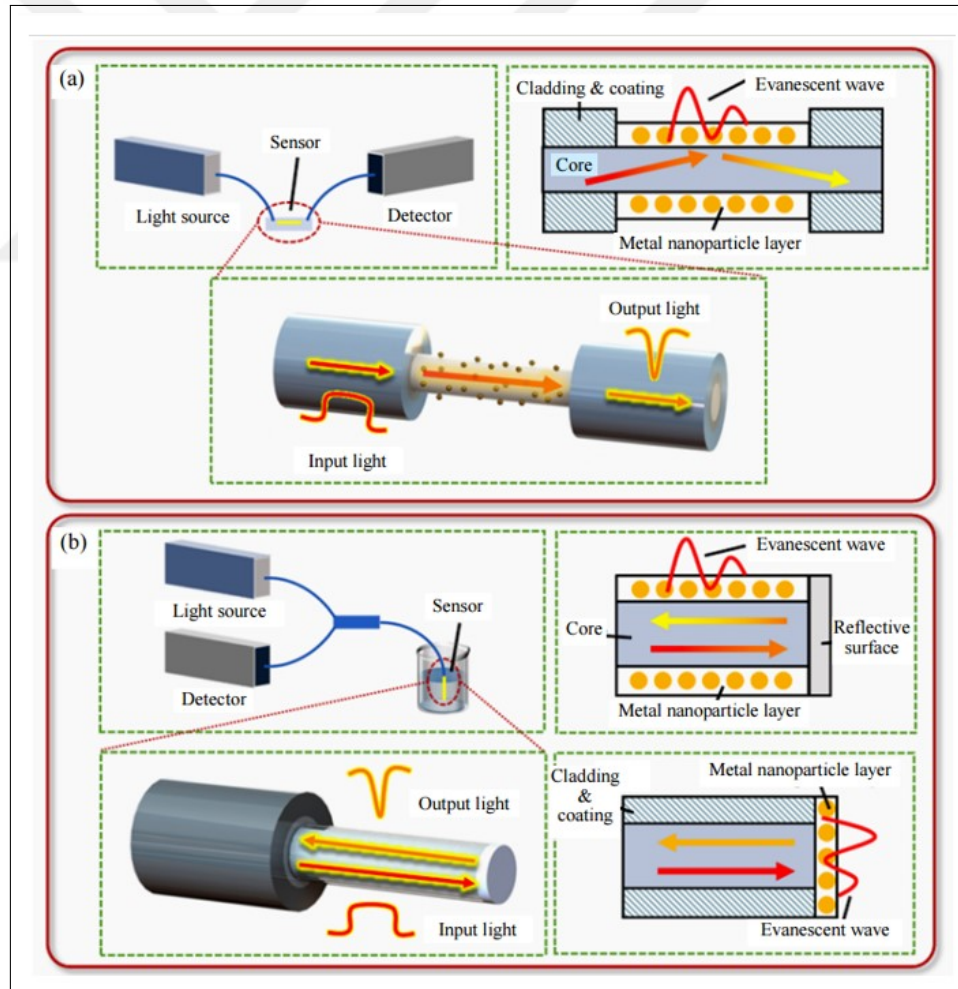


Figure 2.13 (a) transmissive and (b) reflective FO-LSPR sensors [90].

The straight transmissive FO-LSPR sensor (Figure 2.13a) directs light through the fiber core, with LSPR-active nanostructures placed on the outer surface acting as the sensing area. Changes in light intensity or wavelength, caused by interactions along the fiber, are measured at the opposite end. In contrast, the flatheaded reflective configuration (Figure 2.13b) transmits and collects light from the same fiber end using a fiber-optic coupler. Here, nanostructures are positioned on the fiber's end-face or near the probe's end, and the reflected light is analyzed to detect changes. This reflective design is ideal for applications requiring close sample access, such as implantable medical diagnostics, and integrates easily with external systems like spectrometers or detectors. In this thesis, U shaped fiber optic sensors were utilized [figure 2.14] and classified as straight transmissive fiber optic biosensors.

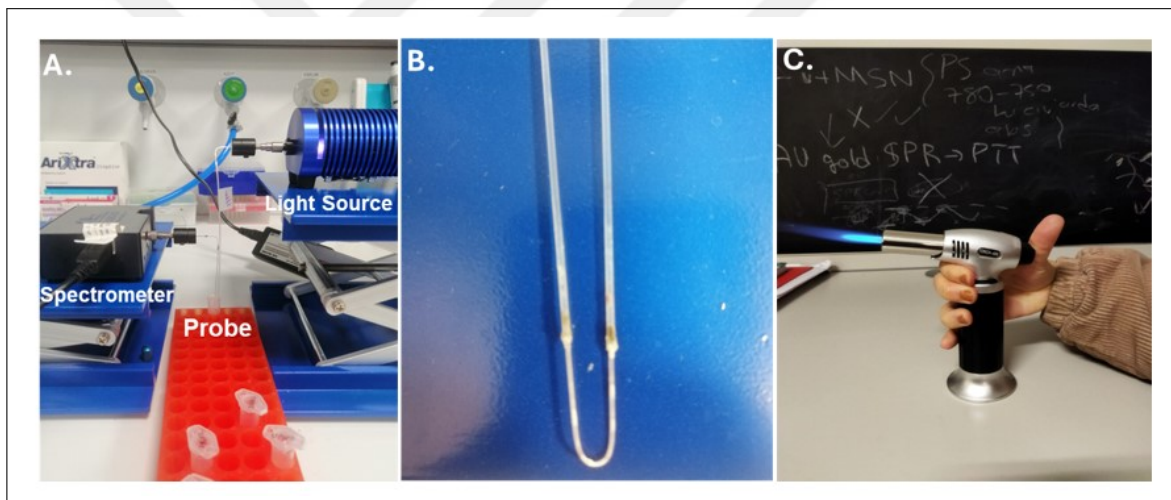


Figure 2.14 (a) Optical set up for measurements (b) U-shaped fiber probe (c) butane flame.

General view for LSPR-based FO sensors was shown in Figure 2.15. This diagram illustrates the key components and set up of LSPR-based fiber optic biosensors, including different geometric configurations (e.g., straight transmissive, flat-headed reflective), optical fiber types (e.g., single-mode, photonic crystal, multi-mode), and fiber probe geometries (e.g., tapered, U-type, Ω -type). Additionally, it highlights the fabrication technologies employed for the sensing region, such as self-assembly of nanoparticles and periodic array nanostructure transfer. The applications of these biosensors are diverse, including the detection of small molecules, microorganisms, nucleic acids, and antigen-antibody immunoassays. This comprehensive view emphasizes the versatility and functionality of LSPR-based fiber optic biosensors.

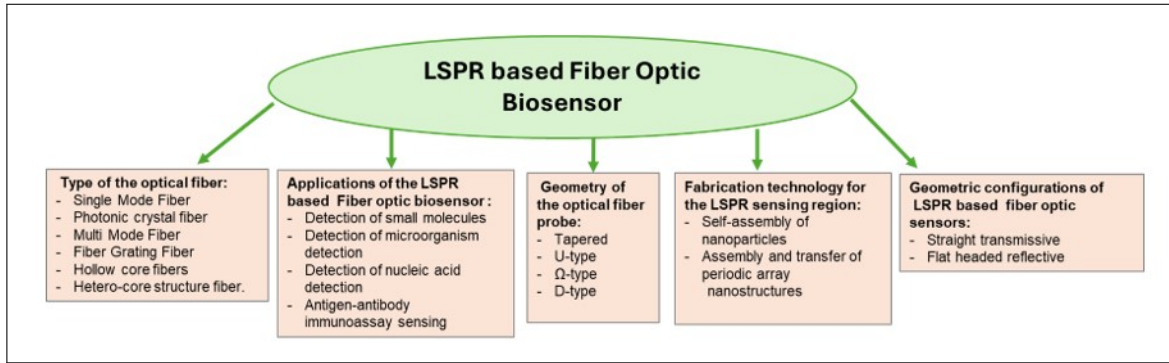


Figure 2.15 General view for Localized Surface Plasmon Resonance based Fiber Optic Biosensor.

As shown in Figure 2.16, a variety of optical fibers have been used in plasmon resonance sensing, including single-mode fibers (SMFs), multi-mode fibers (MMFs), hollow-core fibers (HCFs). As preferred in this thesis as well, MMFs are widely preferred for LSPR sensors because they offer significant advantages over traditional SMF-LSPR sensors. Unlike SMFs, MMFs can propagate multiple light modes, boosting their sensing performance. Additionally, removing the plastic coating from MMFs is a simple process involving physical stripping, which avoids the need for more complicated techniques like acid etching or polishing that could damage the fiber core's surface. This simplicity not only preserves the fiber's integrity but also ensures a more stable sensing structure. The larger core diameter of MMFs further enhances their efficiency by allowing better light coupling and improving the signal-to-noise ratio, ultimately leading to superior overall performance.

Instead of combining different types of optical fibers, modifying the geometric shape of the fiber has gained significant attention as a way to enhance the performance of LSPR biosensors. These sensors are typically classified based on their distinctive shapes, such as tapered, U-shape, Ω -shape, and D-shape. Figure 2.17 provides an overview of these categories along with examples of their various structures.

LSPR based fiber optic biosensors have emerged as a promising tool for biosensing applications. There are numerous applications for LSPR-based fiber optic biosensors, as shown in Table 2.1 [92].

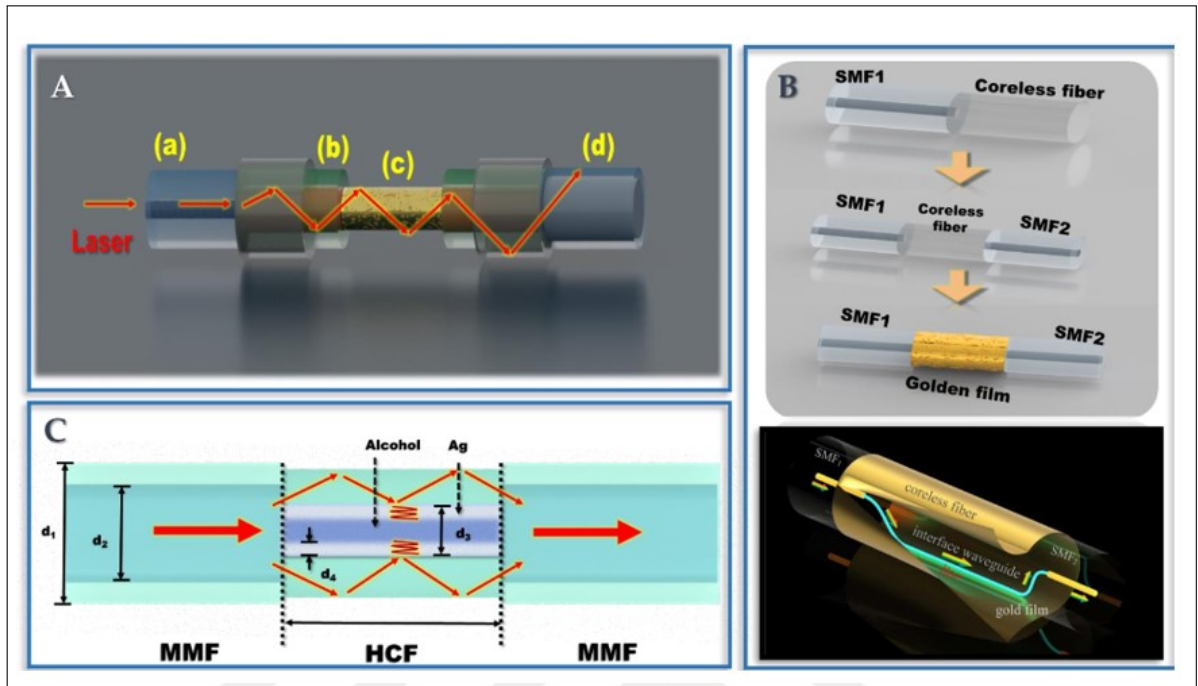


Figure 2.16 Different configurations for fiber optic probes [91].

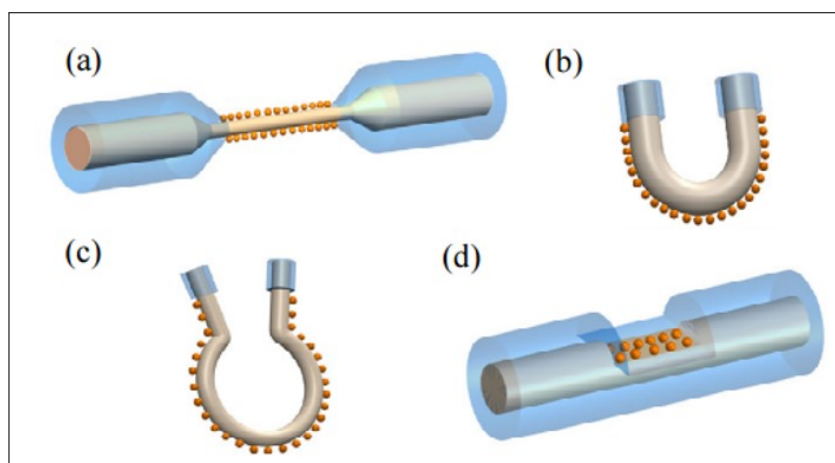


Figure 2.17 Fiber optic biosensors with different shapes: (a) tapered, (b) U-shape, (c) Ω -shape, and (d) D-shape [90].

Table 2.1 Detections with different fiber probe shapes.

Shape	Target	Sensor Performance	Advantage	Disadvantage
Tapered	Glucose	One tapered, sensitivity: 1.06 nm/mM	-	-
	Alanine aminotransferase	Taper-in-taper, Sensitivity: 4.1 pm/(U/L)	-	-
	Ascorbic acid	Four tapered, Sensitivity: 1.1 nm/mM	Ultrarigh sensitivity, superfine diameter	Fragile structure, Complex optical fiber manufacturing method
		Five tapered, Sensitivity: 0.8 nm/mM Eight tapered, Sensitivity: 0.5 nm/mM		
U-type	Cancer cell detection	LOD: 30 cells/mL	Small size, stable structure, simple preparation process	Only transmissive structure
Ω -type	MCF-7 cancer cells	LOD: 12 cells/mL	Small size, 2.5 times higher sensitivity than U-type	Only transmissive structure
	Salmonella typhimurium	LOD: 7.4 CFU/mL		
	MCF-7 cancer cells	LOD: 2.6 cells/mL		
S-type	2,4,6-Trinitrotoluene; 2,4-Dinitrotoluene	LOD: 10 parts per billion (ppb)	1.5 times higher sensitivity than U-type	Fragile structure
D-type	Goat human IgG	LOD: 0.6 μ g/mL	Flexible structure and larger sensing platform	Grinding and polishing surface rough
	2,4,6-trinitrotoluene	Sensitivity: 8.3×10^5 nm/M		
	Water-glycerin solutions	RI sensitivity: 84 nm/RIU		

3. MATERIALS AND METHODS

3.1 Materials

All reagents required for this work were acquired commercially. These included cetyltrimethylammonium bromide (CTAB 99%) and tetra chloroauric(III) acid (extra pure) ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) from Sigma-Aldrich, along with silver nitrate (extra pure) (AgNO_3 , extra pure), sodium borohydride (98%) (NaBH_4), trisodium citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$), high-molecular-weight CH (HW), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), l-ascorbic acid, 11 MUA, and 3-aminopropyltriethoxysilane (APTES) purchased from Sigma-Aldrich. Additional items obtained were ethanol, methanol, sodium hydroxide (NaOH), phosphate buffer saline (PBS) tablets, and hydrochloric acid from Merck Chemicals. Deionized (DI) water was produced using a water purification system.

3.2 The Crafting and Cleansing of the U-formed Fiber Optic Detection Device

A multi-mode fiber optic probe was constructed using an optical fiber featuring a $400\ \mu\text{m}$ diameter core and a rigid polymer cladding with $425\ \mu\text{m}$ diameter (purchased from Thorlabs). The fiber underwent polishing on both ends, and hydrofluoric acid (HF) (48%) was utilized to eliminate the cladding section of the fiber. To create the U-shape configuration for the probe, the etched area was subjected to a butane flame within the 2 cm region's center and then manually bent to achieve the desired curvature. Following this, the fiber probe was immersed in a fresh piranha solution (comprising $\text{H}_2\text{O}_2:\text{H}_2\text{SO}_4$ in a 1:4 ratio) at 60°C for approximately 20 min to eradicate all organic and inorganic impurities. After this treatment, the probe underwent multiple rinses with DI water to eliminate any residual acidic substances. Afterward, the fiber was dried using a stream of nitrogen and put an oven at a temperature of 100°C for 30 min

to generate hydroxyl groups on the glass surface.

3.3 Optical fiber core diameter and sensing length optimization using N-layer matrix method

The optimization, we have the reaction of core diameter and sensing by wavelength interrogation method of SPR based fiber optic sensor using N-layer matrix method.

3.3.1 Theory

In the step index multimode plastic clad silica fiber with fiber core diameter 600 μm and 0.24 (N.A), the middle region of fiber cladding was peeled and coated thin layer of metal. A fiber core coated with metal is further enclosed by the sensing medium with refractive index 1.30.

An incident light from a source is connected into one end of the fiber and transmitted light spectrum is detected at the other end of the fiber. A resonance wavelength depends on the thickness of dielectric constant of the sensing layer, dielectric constant of metal and varying RI of environment medium.

3.3.2 Fiber Core

First layer is made up fiber optic core. The RI of core changes with wavelength according to sellmeier dispersion relation:

$$n_1(\lambda) = \sqrt{1 + \frac{a_1\lambda^2}{\lambda^2 - b_1^2} + \frac{a_2\lambda^2}{\lambda^2 - b_2^2} + \frac{a_3\lambda^2}{\lambda^2 - b_3^2}} \quad (3.1)$$

where λ is the wavelength and where a_1, a_2, a_3, b_1, b_2 and b_3 are sellmeier coefficients. The coefficients are given as $a_1 = 0.6961663$, $a_2 = 0.4079426$, $a_3 = 0.8974794$, $b_1 = 0.0684043\mu\text{m}$, $b_2 = 0.1162414\mu\text{m}$ and $b_3 = 9.896161\mu\text{m}$ [27].

3.3.3 Metal Layer

Second layer is i.e. Au. According to drude model the dielectric constant of a metal is written as

$$\varepsilon_m(\lambda) = \varepsilon_{mr} + i\varepsilon_{mi} = 1 - \frac{\lambda^2\lambda_c}{\lambda_p^2(\lambda_c + i\lambda)} \quad (3.2)$$

Here λ_p is the plasma wavelength and λ_c is the collision wavelength of metal respectively. $\lambda_p = 1.4541 \times 10^{-7}\text{m}$, and $\lambda_c = 1.7614 \times 10^{-5}\text{m}$, for Au [28].

3.3.4 Sensing Medium

Fourth layer is composed of sensing environment whose dielectric constant is denoted as ε_s . If n_s is the refractive index of environment medium, then $\varepsilon_s = n_s^2$. A resonance occurs at the condition when propagation constant of SPW equals to propagation constant of varying light at an incident angle θ .

$$\frac{2\pi}{\lambda}n_1 \sin \theta = \text{RE}\{\text{Ksp}\} \quad (3.3)$$

where, $\text{Ksp} = \frac{\omega}{c} \sqrt{\frac{\varepsilon_m \varepsilon_s}{\varepsilon_m + \varepsilon_s}} = \frac{2\pi}{\lambda} \frac{\varepsilon_m n_s^2}{\varepsilon_m + n_s^2}$. If sensing medium's refractive index is changed, the propagating constant of surface plasmon wave also varies and therefore the resonance occurs at different wavelength. A resonance wavelength varies with a change in RI of environment medium.

3.3.5 Transmitted Power

The reflection coefficient of incident light is expressed by using N layer model [30-33]. It is believed that a collimated source is used to send each guided ray into the silica fiber. The angular power distribution of the rays within the fiber is expressed as:

$$dp \propto \frac{n_1^2 \sin \theta \cos \theta}{(1 - n_1^2 \cos^2 \theta)^2} d\theta \quad (3.4)$$

where “ θ ” is the angle of incidence of light source. The normalized transmitted power of SPR based fiber optic sensor is given Eq.3.5:

$$P_{\text{trans}} = \frac{\int_{\theta_{cr}}^{\pi/2} R_P^{N_{\text{ref}}(\theta)} \frac{n_1^2 \sin \theta \cos \theta}{(1 - n_1^2 \cos^2 \theta)^2} d\theta}{\int_{\theta_{cr}}^{\pi/2} \frac{n_1^2 \sin \theta \cos \theta}{(1 - n_1^2 \cos^2 \theta)^2} d\theta} \quad (3.5)$$

where

$$N_{\text{ref}}(\theta) = \frac{L}{D \tan \theta} \quad (3.6)$$

and

$$\theta_{cr} = \sin^{-1} \left(\frac{n_{cl}}{n_1} \right) \quad (3.7)$$

Number of reflection by ray making an angle θ is denoted as $N_{\text{ref}}(\theta)$. D and L are the fiber core diameter and length of the exposed sensing region. θ_{cr} is fiber's critical angle and n_{cl} is RI of fiber's cladding.

3.4 Functionalization of Fiber Optic Sensor Probe

The sensor probe, treated with piranha solution, was then immersed in a 1% (v/v) APTES solution overnight. Following rinsing with ethanol, the probe was heated to 80°C in a hot air oven for 2 h to create a strong and consistent silane layer. Afterward, the probes underwent a cleaning process involving ethanol and DI water. The probes were dried using nitrogen gas and then subjected to heat at 110°C in an oven for 1 h.

After establishing the amino-silanized layer on the sensor probe, the probe was dipped into an MUA-capped GNR solution for 24 h. Further treatment involved exposing them to an EDC–NHS solution (400 mM:100 mM) for 3 h at room temperature (RT). A solution containing 0.015 g of CH dissolved in 12.5 μ L acetic acid and 1.2 mL DI water was vortexed for 3 h to achieve a uniform solution. The probes treated with EDC–NHS were placed in a CH solution for 24 h. These probes, coated with GNRs and functionalized with CH, were used to detect oligomers. The CH coating durations were implemented for 48, 36, and 24 h, respectively.

3.5 Synthesis of Gold Nanorods

3.5.1 Synthesis of Gold Nanorods Seeds

CTAB-coated seeds were produced through a modified method derived from the seed-mediated technique described by Nikoobakht and El-Sayed. Essentially, the nanoparticles were formed by combining a freshly made ice-cold NaBH_4 solution (0.1 mL, 0.0264 M) with a solution containing HAuCl_4 (0.025 mL, 0.05 M) and CTAB (4.7 mL, 0.1 M). This resultant mixture underwent vigorous stirring for 2 min while kept at a temperature of 28°C. Following its preparation, the solution, exhibiting a faint brown tint, was employed for subsequent experiments an hour later.

3.5.2 Growth of Seeds

After the seeds are made, they are turned into GNRs in the next stage of the manufacturing process. To create a growth solution, a specific sequence of substances is added to a 10 mL aqueous solution of 100 mM CTAB. This includes 530 μ L of 10 mM $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 140 μ L of 10 mM $\cdot \text{AgNO}_3$, 75 μ L of 100 mM l-ascorbic acid, and 2 μ L of 5 M HCl (37%). Once all of these chemicals are combined, 60 μ L of the previously prepared seed solution is quickly added to the growth solution, whereas it is stirred continuously at 28°C. The solution is then left untouched overnight at 28°C.

3.5.3 MUA-capped Gold Nanorods

A technique based on pH was used to remove CTAB completely from the surface of the synthesized GNRs. The process involved the following steps: First, any excess CTAB from GNR solutions with different aspect ratios was removed by centrifugation. Then, the GNRs were suspended in DI water, and a 20 mM MUA aqueous solution was prepared by dissolving 44 mg of MUA in 10 mL of DI water. The MUA solution was sonicated, and a NaOH (0.2 M) solution was added to it while stirring and undergoing periodic sonication until the MUA was dissolved. For the replacement of CTAB present on the GNRs' surface, the MUA solution was stirred for varying durations (2, 4, 6, and 24 h) in the presence of purified GNR solutions.

3.6 Oligomer α -Synuclein Formation

One milligram of recombinant α -synuclein (Millipore, AG938-1MG) was dissolved in a buffer containing 20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, and 1 mM MgCl₂, resulting in a concentration of 1 mg/mL. To eliminate potential fibrils, the proteins were centrifuged at 20,000 g for 20 min at 4°C, and the resulting supernatant was stored at -80°C until further use. The oligomerization process followed a previously described method with some modifications [93]. The proteins were thawed at RT and incubated for 16 h at 37°C without agitation, followed by an additional 12-h incubation at 56°C. Subsequently, the reaction samples were centrifuged at 20,000 g for 20 min at 4°C. The confirmation of oligomerization was achieved through Western blot analysis, ThT testing, and electron microscopy. Monomeric proteins were utilized as a control in these experiments. For the ThT test, in the final reaction, 4.5 μ M concentrations of monomeric and oligomeric α -synucleins were combined with 20 μ M ThT (Sigma-Aldrich, T3516-5G) in PBS. The samples were placed in black wall clear bottom 96-well plates (Corning, 3904) and incubated for 1 h at 37°C with continuous shaking at 400 rpm. The Biotek Synergy HTX Multi-Mode Reader was used to measure fluorescence intensity with excitation set at 440 nm and emission at 485 nm. The fluorescence intensities between the oligomers and monomers were compared.

3.6.1 Western Blot Analysis

Monomers and oligomers were mixed with a 4× sample buffer (Thermo Fisher, B0007) containing 10% beta-mercaptoethanol and boiled for 5 min at 100°C. Following this treatment, the samples were loaded into 4%–12% polyacrylamide gels (Thermo Fisher, NW04120BOX) and transferred onto PVDF membranes (Thermo Fisher, PB5310) using a Trans-Blot Turbo system (Biorad, 1704150). The membranes underwent blocking with 5% skim milk for 1 h at RT. They were then incubated overnight with an anti- α -synuclein antibody (Thermo Fisher, 328100) at a dilution ratio of 1/375, followed by a 2-h incubation at RT with a secondary antibody (Abcam, ab97040) at a dilution ratio of 1/3000. Subsequently, the membranes were treated with a chemiluminescent substrate (Thermo Fisher, 34580), and the resulting signals were captured using the Microchemi 4.2 imaging system (DNR).

3.7 Characterization Methods

3.7.1 Fourier-transform infrared spectroscopy (FTIR)

To investigate how Chitosan (CH) attaches to specially made fiber probes, the FTIR spectra were analyzed using a Nicolet 6700 Thermo FT-IR Spectrometer within the 7800 to 350 cm^{-1} range.

3.7.2 Scanning electron microscopy (SEM)

Images of the probe's surface topography of the fiber optic sensor probe region were obtained using scanning electron microscopy (SEM). To prepare the samples for imaging, a uniform layer of gold with a thickness of 5 nm was prepared. The images were captured using a Philips XL30 ESEM-FEG/EDAX instrument with a voltage of 20 kV and a working mode.

3.7.3 Raman Spectroscopy

Raman spectroscopy measurements were conducted using the Nd: YAG excitation and 0.3 cm^{-1} resolution in the spectral range of 200 nm to 2200 nm cm^{-1} , employing the Renishaw in Via Reflex Raman spectroscopy.

3.7.4 UV-VIS Absorption Spectroscopy

The UV-vis absorption spectroscopy, performed using Varian Cary 100 Bio UV-Vis Spectrophotometer, was used to analyze the optical characteristics of gold nanorods that were synthesized. The absorption spectrum was recorded in the range of 190-900 nm, and a quartz cuvette with a 10 mm path was used for this purpose.

3.7.5 Transmission electron microscopy (TEM)

A transmission electron microscope, JEM 2100 Plus, equipped with a Gatan US4000 CCD, was applied to produce the micrographs of oligomer and monomer protein species. Samples were put on the copper grids coated with formvar/carbon films and stained with a 1% aqueous uranyl acetate solution. Moreover, gold nanorod particles were also characterized using a transmission electron microscope.

3.8 Concentration Analysis

The fiber optic sensor probe region was reacted with various concentrations (10 pM, 20 pM, 40 pM, and 100 pM) of oligomeric α -synuclein protein at room temperature and washed twice with PBS. Then, the reaction was blocked using BSA to remove non specific binding on the sensor surface. The limit of detection (LOD) is calculated from $3*SD/\text{slope}$, detection performance of the sensor was evaluated using SD, the coefficient of variation (CV%), and the accuracy/recovery values.

$$\text{Accuracy/Recovery}\% = \frac{(\text{Calculated mean})}{(\text{Theoretical mean})} \times 100 \quad (3.8)$$

$$\text{Coefficient of variation}\% (\text{CV}\%) = \frac{(\text{SD of sample})}{(\text{Theoretical mean})} \times 100 \quad (3.9)$$

3.9 Statistical Analysis

All data were expressed as mean $\pm$ SD. Comparison among the different groups was assessed by one-way analysis of variance (ANOVA) followed by the Tukey test. A ($p < 0.001$) was used to indicate statistical significance.

4. RESULTS

In this study, CH was coated on MUA-capped fiber probes to improve the sensitivity of the sensors. Various methods, including probe functionalization, nanorod production, and protein formation, were used to characterize the sensor produced and reveal its sensitivity. The results obtained are discussed below.

4.1 Fiber Probe and Gold Nanorods and MUA-capped Nanorod Analysis

The U-shaped multimode fiber probe was fabricated by chemical etching with hydrofluoric acid of the cladding region [94, 95]. The fiber is immersed in a 48% HF solution, ensuring uniform coverage. The HF acid diffuses slowly into the silica lattice, potentially altering the mass of fiber [96]. The thickness of the fiber core, which initially was 404.24 μm , decreased to nearly 267.92-300 μm after chemical etching, as observed in the SEM image in Figure 4.1. By decreasing the fiber thickness, the interaction of the evanescent field with the surrounding medium increased, thereby improving the sensitivity of the sensor to changes in the refractive index (RI) [97]–[99]. Figure 4.2 shows that EDX measurement for fiber probe after coating with chitosan. As seen in elemental analysis, The cross-sectional EDS analysis of the fiber probe coated with chitosan highlights the distinct presence of elements associated with the chitosan layer in Figure 4.2A. Carbon (C) constitutes 31.87% by weight, nitrogen (N), a key component of chitosan, appears at 2.44% by weight, and oxygen (O) contributes 32.6% by weight. Silicon (Si), originating from the fiber substrate, is present at 32.77% by weight. Also, in Figure 4.2B shows that N value increase means that chitosan on the surface. The distribution of elements confirms the successful deposition of chitosan, as evidenced by the nitrogen signal and the increased carbon content compared to the uncoated fiber. The oxygen and silicon signals reflect contributions from both the chitosan coating and the underlying fiber material. This analysis validates the chitosan

coating process and provides insight into its elemental composition.

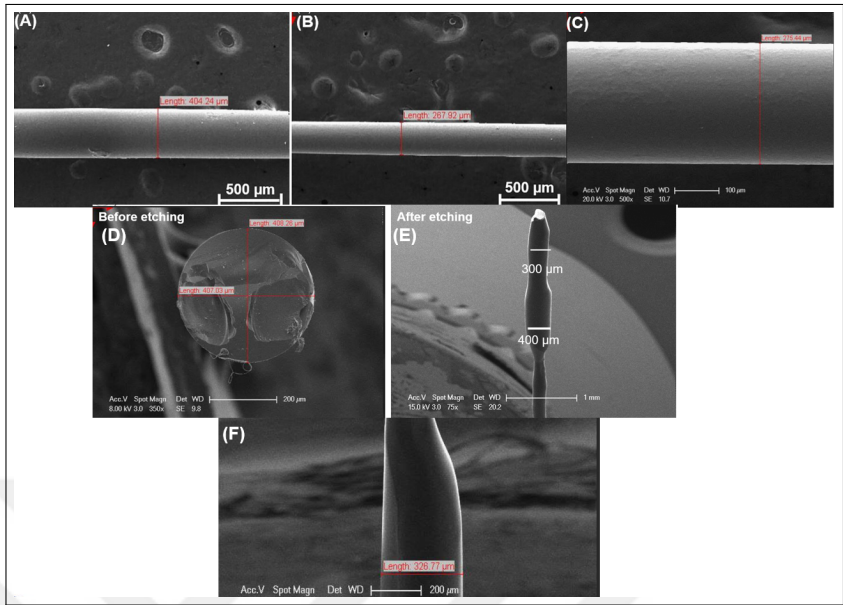


Figure 4.1 Scanning electron microscopy imaging of the fiber probe (A), (D) before HF etching and (B), (C), (E) and (F) after HF etching.

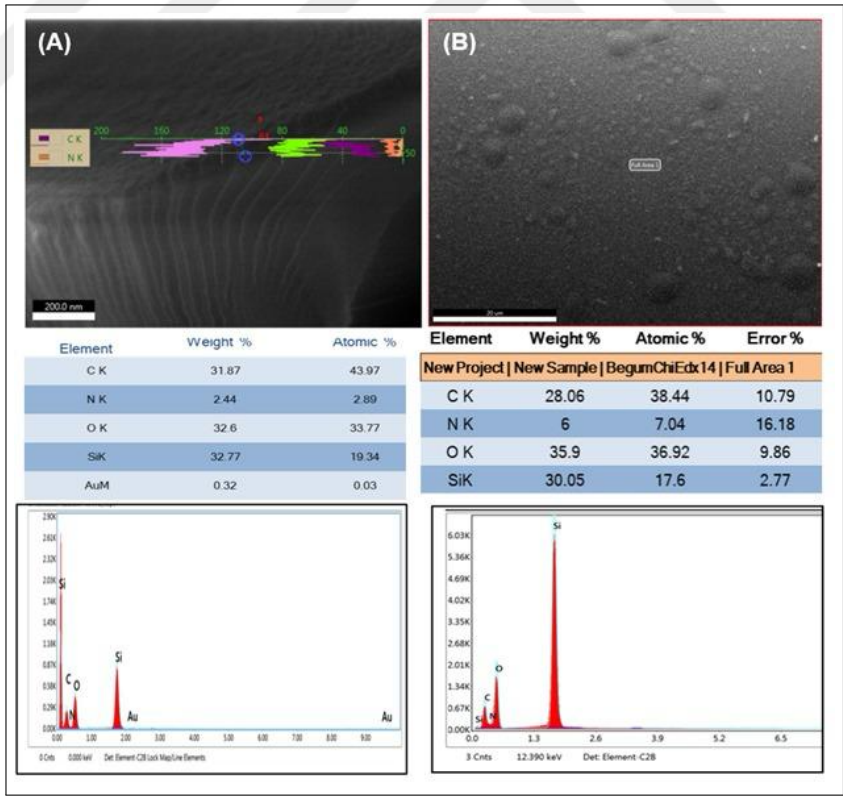


Figure 4.2 EDX Measurement for (A), (B) after coating surface with chitosan.

The comparative EDS measurements between Figure 4.2 clearly show the effectiveness of chitosan deposition. The presence of nitrogen and increased carbon content

in the coated sample are clear indicators of the chitosan layer, while silicon and oxygen remain constant as they belong to the fiber substrate. These findings clearly allow the use of chitosan coatings for functional modifications of fiber surfaces.

Compared with uncoated fibers, GNR-coated fibers are in a purple color after the coating process. The typical purple color from Figure 4.3 is due to the natural optical characteristics of GNRs and the homogeneous deposition on the surface of the fiber. For its part, the uncoated fibers maintain their white and colorless appearance due to the absence of GNRs. This optical difference offers a practical way of ensuring the accomplishment of coating and checking if the GNRs were effectively bound to the surface of the fiber.

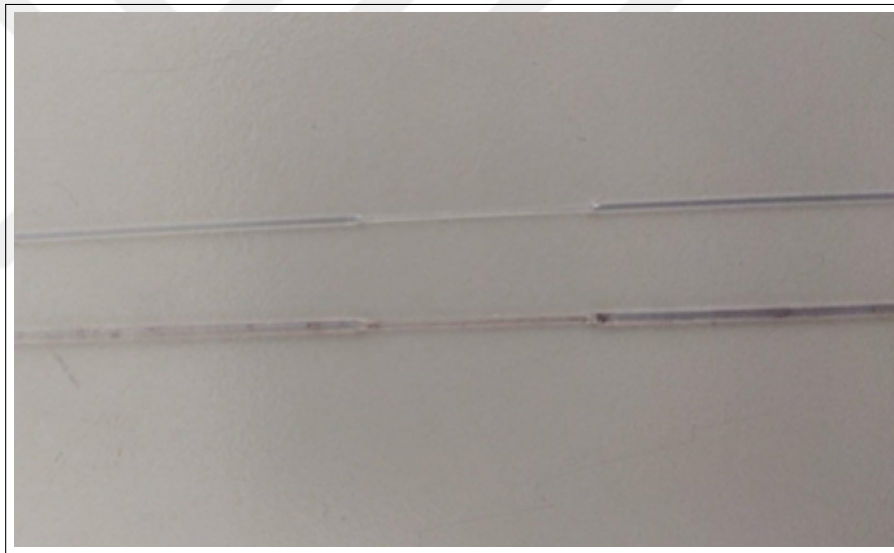


Figure 4.3 FO probe without Gold Nanorods (above), FO probe with Gold Nanorods (below).

Morphological and structural observations were obtained using Scanning Transmission Electron Microscopy (STEM). Accordingly, GNRs and MUA-capped GNRs were prepared using multiple steps, the MUA-capped GNR overnight mixed to construct the immobilization matrix. The synthesized MUA-capped GNRs were immobilized on the sensor probe surface after being treated with APTES solution and the morphological changes were observed by using Raman and SEM in Figure 4.4 and Figure 4.5, respectively.

The aspect ratio (AR), which is the ratio of width to height of GNR, can be determined using the maximum absorption from the spectrum. The Eq. 4.1 shows the relation between AR and L-SPR absorption maximum ($\lambda_{\max}$) [100].

$$AR = \frac{(\lambda_{\max} - 478)}{96} \quad (4.1)$$

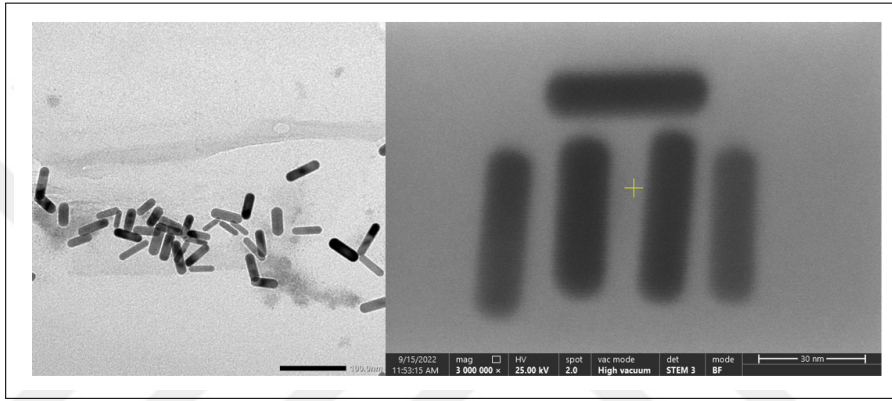


Figure 4.4 Characterization of Gold Nanorods (GNRs). (A) STEM image of well-dispersed Gold Nanorods (B) STEM image of MUA-capped GNRs.

Due to instability of CTAB bilayer, the passivation of the nanorod surface by MUA ligand takes place [101]. Parameters like pH value and stirring time are essential for synthesizing MUA-capped GNRs. SEM images demonstrate GNR-MUA complexes' dispersion. This shows no substantial changes in their morphology compared to the initially synthesized MUA-capped GNRs under pH 10. The stability of MUA-capped GNR prepared in a basic environment and its homogeneous attainments have also been observed in other studies [102, 103]. As shown in Figure 4.5(A,B), it is evident that MUA-capped GNRs (stirred for 24 h at pH 10) on the sensor probe surface maintained homogeneous distribution. At lower pH values (stirred 4 h at pH 5 and), the increase of interactions leads to aggregation of MUA, which can be observed in Figure 4.5C.

The absorption spectra depicted in Figure 4.5D showcase distinct plasmon bands for both GNRs and MUA-capped GNR solutions. Notably, the T-SPR appears near 530 nm, whereas the L-SPR peaks around 774 nm for GNRs. The MUA-GNR spec-

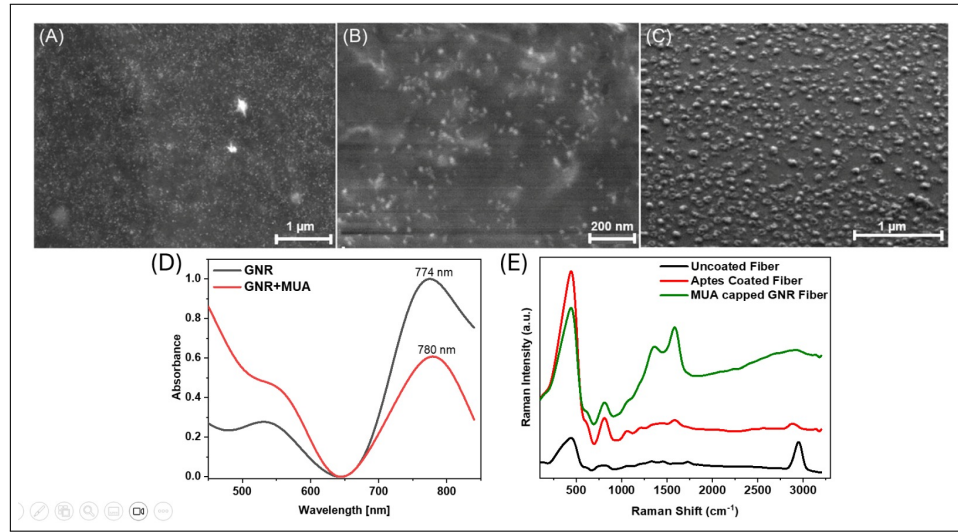


Figure 4.5 SEM images of fiber probe surfaces with (A,B) non-aggregated MUA-capped GNRs with pH 10 (C) aggregated MUA-capped GNRs with pH 4 and pH 5, (D) Normalized UV-visible spectra of GNR (black line) and MUA-capped GNR (red line) occurrences were observed after 24 h stirring. After GNRs were coated with MUA, the wavelength shift was monitored from 774 nm to 780. (E) Raman spectra of sensor probe for bare fiber (black), APTES coated (red), and MUA coated fiber (green).

trum showed a redshift from 774 to 780 nm in the L-SPR peak because of surface modification, and the T-SPR showed from 530 to 540 nm. In the ligand exchange process, thiols attach to gold surface through Au-S bonds, reducing the number of free electrons of GNR. Increasing electron density results in a higher Surface Plasmon Resonance frequency; thus, the SPR shifts to the redshifted region of the spectrum the electron density is decreased [104, 105]. The L-SPR shift is attributed to alterations in the RI at the GNRs' surface, resulting from the ligand exchange. Previous research reveals that L-SPR exhibits greater sensitivity to variations in the RI within the surrounding environment of GNRs than T-SPR [106, 107]. These findings substantiate prior reports, affirming the heightened sensitivity of GNRs with increased aspect ratios. Figure 4.3E shows Raman analysis which was conducted to evaluate the bonding of GNRs coating on U-shaped probes, both before and after the layer with MUA-capped GNRs. The surface functionalization process on the fiber surfaces was achieved via a self-assembly method utilizing APTES monolayer formation. This process was initiated by the piranha treatment, which introduced abundant surface hydroxyl groups (-OH) on the fiber samples through a hydroxylation process. A distinctive peak be-

tween 2900 and 3010 cm^{-1} on the uncoated fiber surface indicates the existence of -OH bonds on the surface. Subsequently, during the silanization step, APTES was formed on the fiber surface, showcasing various atomic bands in the vertical direction. Notably, specific vibrations were observed at 327 and 1605 cm^{-1} , as indicated [108]. APTES-coated fiber, the peak at 750 cm^{-1} and 1075 cm^{-1} corresponds to the C-H bonds and silane structure of APTES. The thiol (SH) groups present strong adherence to the GNR surface by forming a covalent metal-sulfur bond, thereby creating a well-ordered, self-assembled monolayer. The process involved the addition of an aqueous GNR particle suspension to a solution containing this alkane and ethanol. Various vibronic modes of the hydroxyl groups (around 2900 cm^{-1}) and the end groups of the thiol molecule (about 750 cm^{-1}) were observed in the Raman spectrum. The peak at 750 cm^{-1} reflects S-C bonds from binding thiol (-SH) groups of MUA to the gold surface [109]. C-H stretching vibrations at 1300 cm^{-1} and 1571 cm^{-1} confirm the ordered structure of MUA. The addition of MUA capped GNR to APTES surface led to hyperchromic effect (Mohammad E. Khosroshahi et. al), increasing Raman peak intensities.

4.2 Simulation for Fiber Optic Sensor Probe and Optimization

In this study, we investigated multimode optical fibers with various core diameters in the range from 100 μm , 200 μm , 300 μm in a length of 0.5, 1, 2, and 3 cm in the sensing region to evaluate their performance in transmitting modes in Figure 4.6. Using simulations, results show that the core diameter and sensing region length significantly affect the optical transmission efficiency. Among the configurations tested, the best balance was always found with the 300 μm core diameter-fiber in minimizing optical losses and a high transmission intensity in the working wavelength range. Smaller core diameters (100 and 200 μm , respectively) suffered with a pronounced loss mostly due to scattering and limited mode confinement.

The present study revolves around investigating multicomponent multimode optical fibers whose core diameters range from 100 μm to 200 μm , 300 μm in the

measurement lengths of 0.5 cm, 1cm, 2 cm, and 3 cm. The transmission performance of these modes is evaluated. The simulation results show that both core diameter and sensing region length significantly affect optical transmission efficiency. Among the tested configurations, the fiber with a 300 μm core diameter always demonstrates the best balance concerning minimizing optical losses and high transmission intensity within a studied wavelength range. Yet on the contrary, small core diameters like 100 and 200 μm will find a significant loss due to scattering and limited mode confinement.

The size of the sensing region is also an important parameter that determines the transmission efficiency. Smaller sensing regions, for example, 0.5 cm and 1 cm, have lower losses as the interaction of light with the environment is less. Longer sensing regions are, however, required in practical applications for sensing. Among various alternatives, that is why the length of the sensing region was standardized to 2 cm in this study, finding the best compromise between sensitivity and transmission efficiency. The other length of 3 cm for the sensing regions caused more losses since the interaction length was greater, thus increased scattering and absorption effects were well manifested. The combined significance of core diameter and sensing region length thus shows that the configuration of a 300- μm core with a 2-cm sensing region is the best, offering high energy delivery without compromising in practical application.

The results thus indicate the careful optimization of both geometric parameters and length of sensing regions to optimize the potential of multimode optical fibers for use in sensing applications. They indeed form a study basis for the specification of fiber configurations designed specifically for operation, thus ensuring maximum efficiency and reliability. For the optimization etching time, power and absorbance measurement were applied to fiber probe in Figure 4.7. In Figure 4.7A shown that power was higher in air before etching around 4 mW. When fiber probe interact with the HF, power starts to decrease and after 30 min power was around 2.3 mW. Power loss also was observed in between 60 min-180 min. After 180 min etching time, absorbance value dropped in Figure 4.7B. It means that optical transmission capability of the fiber probe stopped. Therefore, 120 min etching time selected as an optimum time.

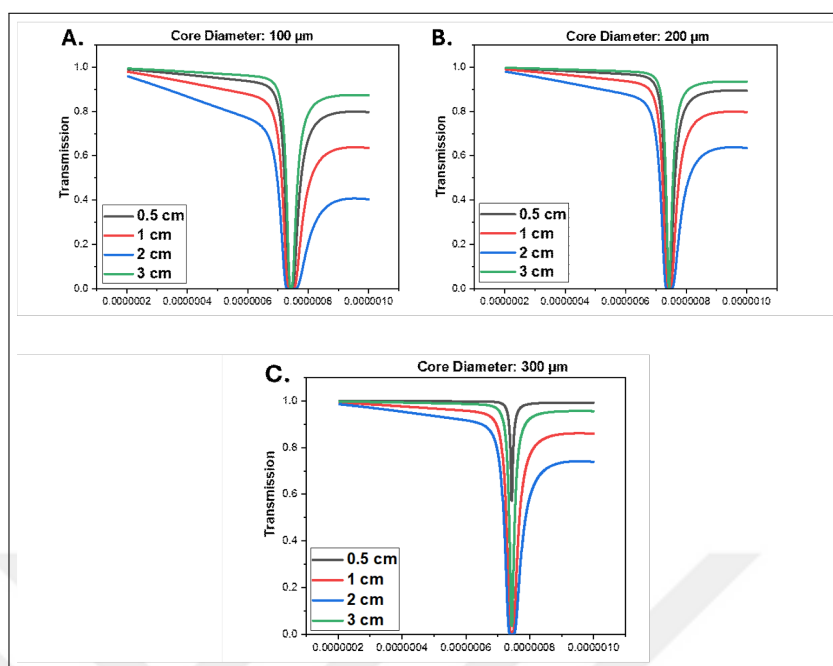


Figure 4.6 Transmission spectra for fiber core diameter A) 100 μm , B) 200 μm , C) 300 μm with 0.5 cm, 1 cm, 2 cm and 3 cm sensing region.

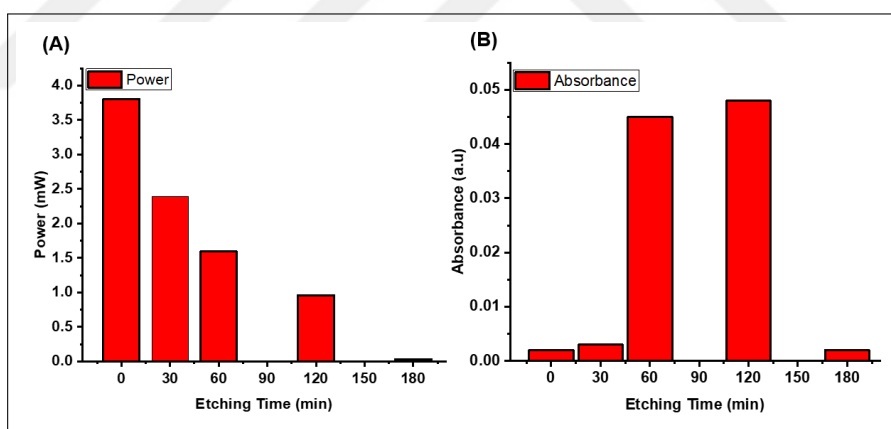


Figure 4.7 Fiber probe etching time optimization (A) power measurement with different etching time (B) Absorbance measurement for different etching times.

4.3 Sensor Probe Analysis

The chemically treated MUA-capped GNRs immobilized probe underwent a process to establish a stable CH film. Figure 4.8 presents SEM images of the thickness of CH-coated on the fiber probe and the analysis conducted using FTIR. The resulting CH film thicknesses were measured at 75 ± 4 nm after 24 h (Figure 4.8) and 122 ± 3 nm after 36 h (Figure 4.8B), and by using SEM. The sensor probes were incubated

in CH solution for 24h and 36 h, respectively. FTIR spectroscopy was utilized to illustrate the binding of CH onto the sensor probe. Figure 4.8C displays the FTIR spectra of the fiber probe surfaces, depicting the spectra black (before) and red (after) the functionalization with CH. Through FTIR spectroscopy analysis, the chemical composition of the fiber probe surfaces before and after CH modification was characterized. Upon CH functionalization, the FTIR spectrum of the fiber optic sensor probe region exhibited distinctive features. A notable transmission dip was observed around 3256 and 3391 cm^{-1} , which signifies the overlapping O–H and N–H stretching vibrations [110]. Additionally, vibrations observed at 1550 and 1651 cm^{-1} correspond to NH₂ bending/amide I band and C=O, respectively. An important observation was the absence of the N–H stretching at 3256 cm^{-1} in the spectrum obtained for optical sensor probes solely coated with GNRs. This absence confirms the cross-linking of CH, further confirming its successful binding and presence on the fiber probe surface [111].

4.4 Optimization of Oligomer α -Synuclein

The transition of α -synuclein from its monomer state to an oligomer form plays a critical role to better understand the molecular events driving neurodegenerative diseases, such as Parkinson’s disease. Given the importance of studying oligomers, we designed and conducted a series of optimization experiments to determine the ideal conditions for inducing α -synuclein oligomerization while minimizing fibril formation. This section outlines the challenges faced during the optimization process, the approaches undertaken, and the outcomes observed, ultimately leading to the identification of conditions that yielded robust oligomers. Initially, monomer α -synuclein was maintained at 37°C for 16 hours, followed by an additional 6-hour incubation at 56°C. This approach aimed to mimic previously reported protocols for promoting oligomerization. However, when we analyzed the samples using western blot (WB) (Figure 4.9), no oligomeric bands were detected, suggesting that the conditions were insufficient to drive the oligomerization process. This was a disappointing result but provided us with important insights: the incubation time might be too short to allow for oligomer formation.

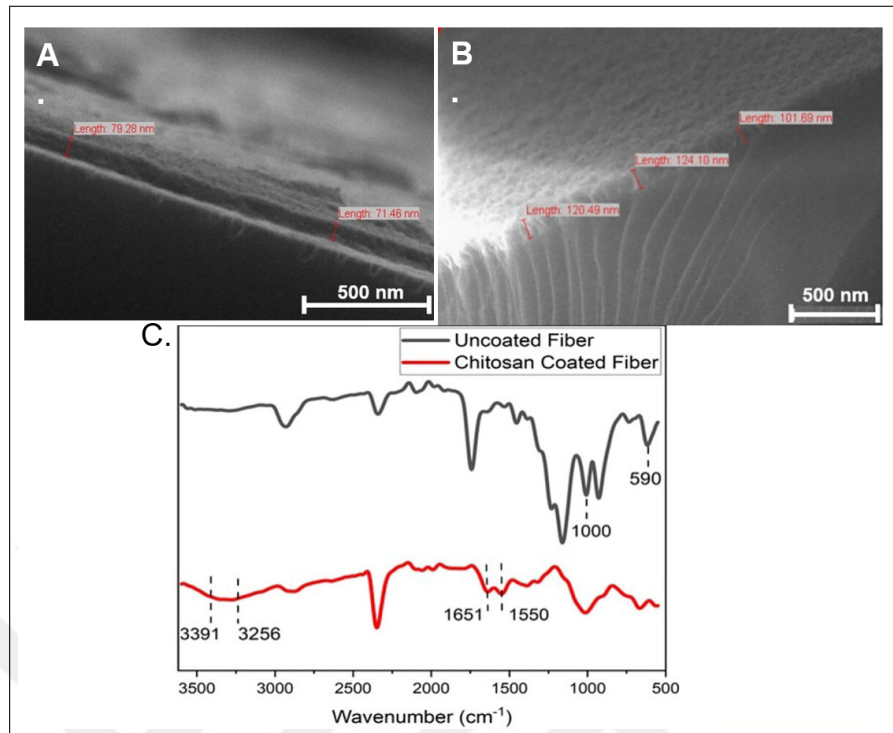


Figure 4.8 Cross-sectional scanning electron microscopy (SEM) images of the probe showing chitosan film thicknesses after (A) 24 h (B) 36 h, C) FTIR spectra of sensor probe immobilized with gold nanorods: bare fiber probe (black line) chitosan coated fiber probe (red line) in the 500 cm⁻¹ and 4000 cm⁻¹ spectral ranges and chitosan chemical composition is presented through spectral ranges between 3256 cm⁻¹ and 3391 cm⁻¹, other vibrations obtained at 1550 cm⁻¹ and 1651 cm⁻¹ represent NH₂ bending/Amide I band & C=O, respectively.

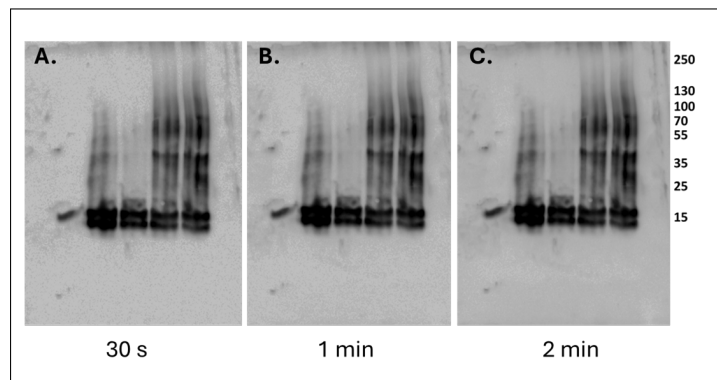


Figure 4.9 Western blot analysis of α -synuclein after incubation at 37°C for 16 hours followed by 6 hours at 56°C. Membranes were analyzed using chemiluminescent detection with varying exposure times of A) 30 seconds, B) 1 minute, and C) 2 minutes. Despite different exposure times, no oligomeric bands were observed, indicating that the incubation conditions were insufficient to promote oligomerization under these experimental settings.

In the next step, we extended the initial incubation at 37°C from 16 hours to 24 hours, keeping the secondary incubation at 56°C for 6 hours unchanged. This adjustment aimed to allow more time for the monomers to interact and initiate the oligomerization process. Interestingly, under these conditions, we observed the presence of fibrils alongside the monomer bands (Figure 4.4). While this indicated some degree of structural change in the protein, the simultaneous appearance of fibrils suggested that the balance between oligomer and fibril formation might be a delicate one.

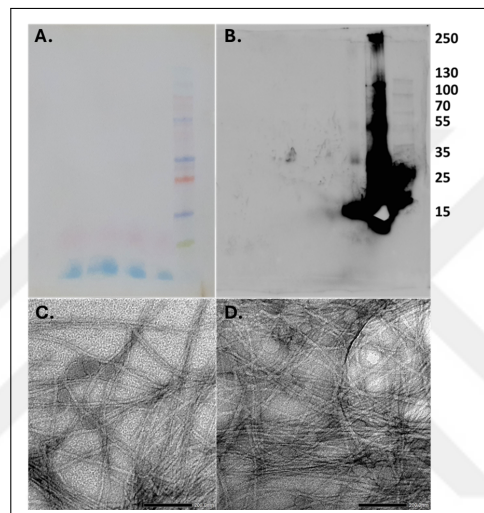


Figure 4.10 Western blot analysis of α -synuclein after incubation at 37°C for 16 hours followed by 6 hours at 56°C. Membranes were analyzed using chemiluminescent detection with varying exposure times of A) 30 seconds, B) 1 minute, and C) 2 minutes. Despite different exposure times, no oligomeric bands were observed, indicating that the incubation conditions were insufficient to promote oligomerization under these experimental settings.

Determined to identify the ideal conditions, we made further adjustments to the protocol. Specifically, we returned to the 16-hour incubation at 37°C but extended the secondary incubation at 56°C to 12 hours. Remarkably, this modification proved successful. Western blot analysis (Figure B) revealed distinct oligomeric bands, confirming the presence of oligomers. Furthermore, no significant fibril formation was observed, which was a crucial outcome for our study. To further validate the presence and stability of the oligomers, we performed Thioflavin T (ThT) fluorescence assays and transmission electron microscopy (TEM). ThT is an amyloid-binding dye that initiates strong fluorescent signals when bound to amyloid fibrils. The ThT results demonstrated there are no fibrils in monomeric and oligomeric samples in Figure A and B, while TEM im-

ages (Figure C) provided direct visualization of oligomeric particles, confirming that the aggregates formed under these conditions were predominantly oligomers. Otherwise, Western blot and electron microscopy analysis verified the oligomerization by molecular weight. Western blot analysis showed that the oligomerization samples had higher molecular weight complexes besides monomers. The findings of electron microscopy supported the ThT test and Western blot analysis. This optimized protocol represents a significant breakthrough for our research, as it reliably produces oligomers without fibrils, making it suitable for studying the biochemical and structural properties of oligomeric α -synuclein. The findings not only emphasize the importance of incubation time and temperature but also illustrate the fine balance required to direct monomers toward oligomerization rather than fibrillation. Through careful optimization and persistence, we were able to overcome the challenges faced during the initial stages of this work. In conclusion, the development of this protocol has provided us with a reliable method to generate and study α -synuclein oligomers. These oligomers were successfully characterized using WB, ThT, and TEM, confirming their reproducibility and robustness.

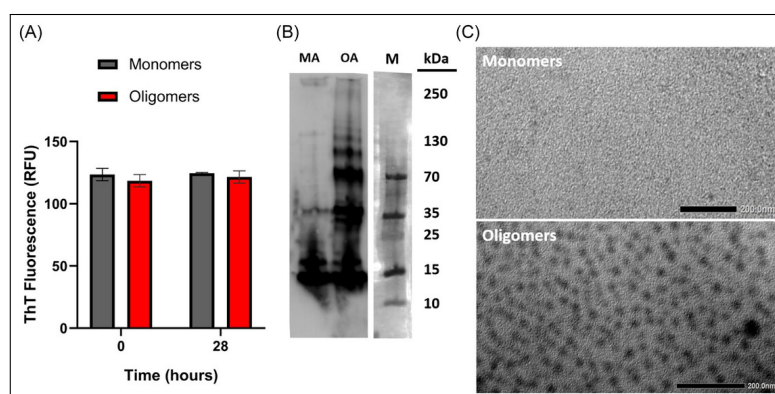


Figure 4.11 Verification of oligomerization. (A) The graph displays the fluorescent intensity of monomers and oligomers at the initial (T0) and final (T28) stages of the reaction. The samples exhibited similar and non-increasing fluorescent intensities, indicating that oligomerization did not induce fibrillization. (B) A Western blot comparison between oligomerized samples and monomers revealed the presence of higher molecular weight structures in oligomerized samples. The marker of this membrane was visualized separately using colorimetric imaging. (C) Transmission Electron microscopy (TEM) analysis demonstrated that oligomerized samples (below) formed larger structures than monomers (above), supporting the findings from the ThT test and Western blot. (MA: monomeric α -synuclein, OA: oligomeric α -synuclein, M: marker).

4.5 Sensitivity for Fiber Optic Sensor Probe

PBS buffer was evaluated to determine the sensitivity and the limit of detection of sensor. The sensor probe, featuring a CH film and a matrix of MUA-capped GNRs, underwent exposure to diverse RI solutions to evaluate the influence of the CH film on the LSPR response. Variations in the solution's RI around the fiber-optic sensor, coated with the nanoparticle matrix, can alter the LSPR characteristics, thereby inducing changes in absorbance. In our experiments, glucose solutions with concentrations of between 20 and 90 mM in DI water were prepared, and glycerol was added to further increase the RI, resulting in the range of 1.373–1.395. Figure 4.12A representative illustrates the absorbance spectrum observed for the optical sensor probe modified with gold nanorods. The alterations in absorbance concerning different RI are delineated in Figure 4.12B. Upon thorough analysis, the calculated average sensitivity for these variations was determined to be $502.69 \Delta\lambda/RIU$.

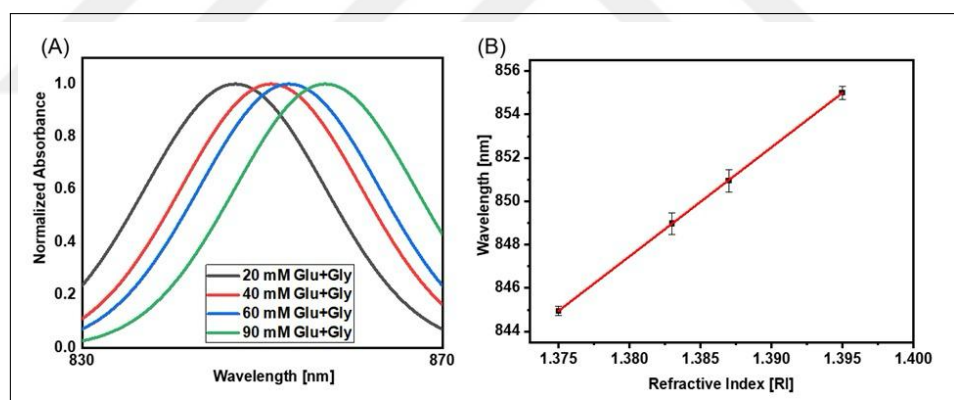


Figure 4.12 Representative graphics depict a nanostructured sensor's light absorption properties for measuring variations in refractive index. A) absorbance changes for MUA-capped GNR coated probe across a range of refractive index values. B) LSPR wavelength against the refractive index for a similar sensor using nanorod.

4.6 Absorbance Measurements for Oligomer α -Synuclein

The proposed apparatus detects the oligomer form of α -syn by using CH. Given the GNRs' sensitivity in detecting oligomeric α -synuclein, the biosensing experiment

was conducted at a stable RT of 25°C in Figure 4.11. Figure 4.12 illustrates the absorbance spectra corresponding to concentrations of 10, 20, 40, and 100 pM of oligomer α -syn introduced into PBS. Notably, concentrations of oligomer α -syn in the cerebrospinal fluid of PD patients typically range from 1 to 10 pM [112]. Accordingly, the CH-coated fiber optic probe was exposed to lower concentrations of OAs (10-100 pM). In Figure 4.13A, interestingly, lower oligomer concentrations demonstrated a proportional increase in the shift of peak absorption wavelength with rising OA concentration. However, a similar trend was not observed for absorbance values, identical to the detection of oligomer α -syn protein in PBS. Specifically, upon binding 10 pM oligomer α -syn, a redshift of 0.3 nm was detected, whereas a shift of 1.2 nm was observed with 100 pM oligomer α -syn protein.

The increase in concentration of the OA solutions was observed to be associated with a redshift in resonant wavelength. This shift in resonance wavelength was attributed to the increase of effective refractive index (RI) in the sensing region close to the plasmonic metallic layer [113]. In contrast, there was no observable wavelength shift in the control experiment using α -synuclein-free PBS. Analyzing the variation of peak absorbance wavelength shift against oligomer α -syn concentration through regression analysis revealed linearity with an R² value of 0.968 (depicted in Figure 4.13B). The calculated LOD was determined to be 11 pM. The observed LOD is like that reported in a previous study using oligomer α -syn protein [114]. Impressively, the proposed device enabled rapid diagnosis of α -syn oligomers within 15 minutes significantly quicker than conventional biosensors. This highlights the potential of the developed LSPR biosensor in PD diagnostics, with promising applications in point-of-care analyses.

The analysis of absorbance values for sample concentrations of 10 pM, 20 pM, 40 pM, and 100 pM in both phosphate-buffered saline and mimicked serum environments were done in Figure 4.15. The negative controls in both environments exhibited low absorbance (~ 0.02 and 0.05 a.u.), signifying the system introduced little background signal and results could be relied on. Furthermore, absorbance increased systematically with concentration in each setting, showcasing the system's quantitative detection capabilities. A statistically significant relationship between target molecule concentra-

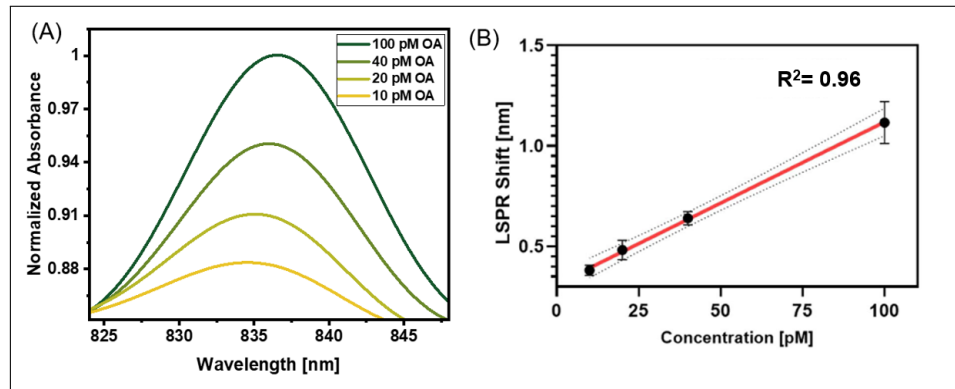


Figure 4.13 Absorbance values for oligomer alpha synuclein in PBS. (A) LSPR spectra of chitosan functionalized biosensor probe after treatment with PBS solutions having varying oligomer α -syn concentrations, (B) Concentration versus LSPR shift plot. Curve fitting was performed using a linear fit equation on GraphPad Prism. The figure shows the best-fit line (solid) within the confidence band (dashed).

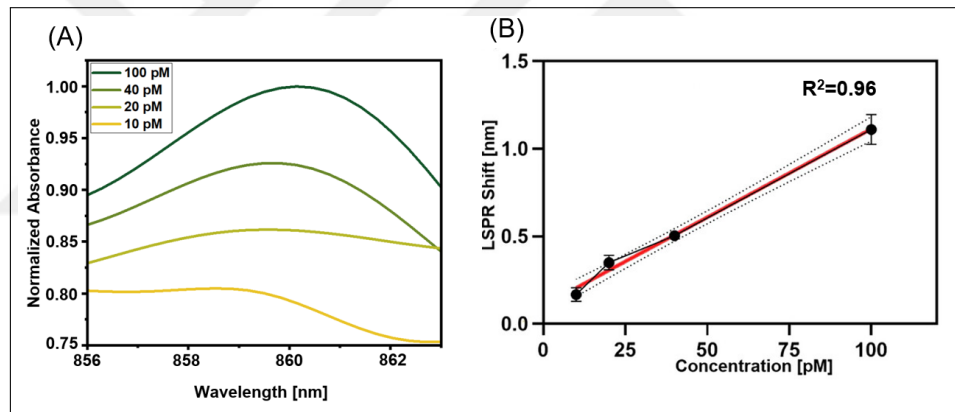


Figure 4.14 Absorbance values for oligomer alpha synuclein in mimicked serum sample with PBS+1.5% BSA. (A) Absorbance spectra of chitosan functionalized biosensor probe after treatment with mimicked serum sample solutions having varying oligomer α -syn concentrations, (B) Linearity plot of α -syn oligomer fitted with linear parameter equation on GraphPad Prism from the output of absorbance spectra. Curve fitting was performed using a linear fit equation on GraphPad Prism. (OA: Oligomer α -synuclein).

tion and absorbance was observed in both environments ($p < 0.001$). These results demonstrate the reliability of the measurements obtained in both PBS and mimicked serum and highlight the system's capability to operate under different environmental conditions.

These results show that chitosan can detect oligomer form of alpha synuclein

in both environments. The serum mimicked environment is also reliable, and it shows that this sensor system has potential in biological environment. As future study, this sensor system can be applied in real serum samples.

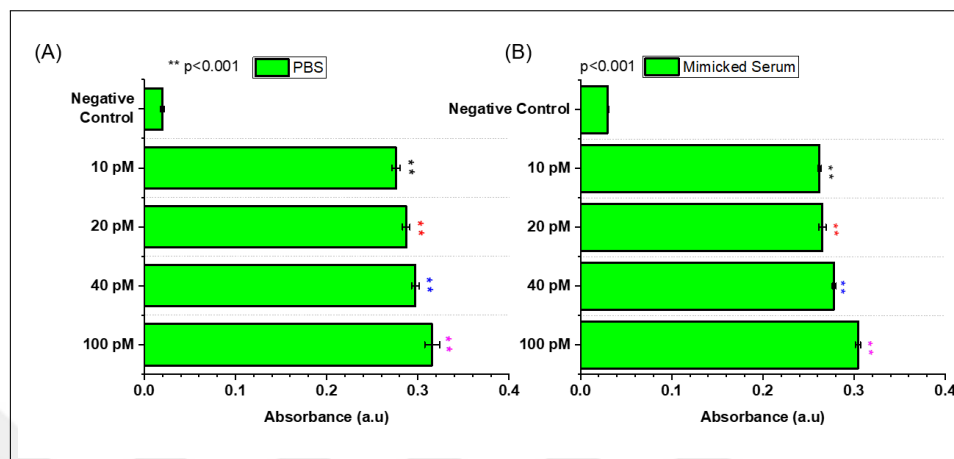


Figure 4.15 Statistic Analysis for negative controls 10 pM, 20 pM, 40 pM, 100 pM level concentrations (A) in PBS (B) in mimicked serum samples (OA: Oligomer α -synuclein). ** $p < 0.001$ * indicates statistical significance compared to the control sample. Data represent mean values $\pm$ SD of triplicate experiments ($n = 3$).

4.7 Concentration Analysis for Alpha Synuclein

Oligomeric α -synuclein quantification in this study was with PBS as matrix and consecutive spiking at concentrations of 10 pM, 20 pM, 40 pM and 100 pM to assess method performance (Figure 4.16). PBS was selected as a simplified and defined buffer system to avoid matrix effects typically present during quantification with complex biological fluids. It was evaluated in terms of two main performance parameters: accuracy expressed as recovery within the 80-120% range and precision indicated by the coefficient of variation (CV) which was taken acceptable if it was less than 20%. In Figure 4.16A, This trend indicates that the method is sensitive to changes in α -synuclein concentration within the measured range. The method was linear as evidenced from plotting of the calculated concentrations vs the nominal spiked concentrations (Figure 4.16, right). The data showed a high linear correlation ($R^2 = 0.9994$), demonstrating good alignment with the individual measured and theoretical values. The high R^2 value reflects the robustness of the method and its ability to produce

reliable results across a wide dynamic range. Recovery values were calculated to determine the method's accuracy, with all concentrations meeting the predefined acceptance criteria of 80-120% recovery. The recovery rates were 109.6% (10 pM), 96.3% (20 pM), 99.6% (40 pM), and 94.7% (100 pM). These results validate the method's accuracy for low to high concentration ranges. All CV values were below the 20% threshold, indicating the method's reproducibility, and the precision as CV was also assessed. The 10 pM CV value was highest at 2.39%, with lower variability seen for 20 pM (8.81%), 40 pM (7.4%) and 100 pM (15.7%), showing reliability at low concentrations.

PBS also served as the matrix, creating a controlled and interference-exclusion setting for α -synuclein detection. Unlike complex matrices, such as serum, the use of PBS enabled us to assess the sensitivity, linear range and other performance characteristics of the method with the interference from other proteins or matrices removed. This is especially useful in reducing the variability of the absorbance values reflecting the concentration of the spiked α -synuclein in a complex sample environment, to improve the robustness and reliability of the method.

Overall, the method had good accuracy and precision (recovery values between 80–120%) with CV values always lower than 20%. The high linear correlation ($R^2 = 0.9994$) also confirmed the robustness of the method for quantifying oligomeric α -synuclein. Results from this study confirm the suppressive effect of PBS as a matrix provides a consistent, interference-free environment for α -synuclein detection which can be used as a basis in the future development and optimization of such assays in more complex biological matrices.

According to the results shown in Figure 4.17, Quantitative analyses of oligomeric α -synuclein proteins at different concentrations (10 pM, 20 pM, 40 pM, and 100 pM) were carried out in this work, and the results were assessed in a serum mimic environment. Serum biological characteristics were simulated using a PBS + 1.5% BSA solution. This combination mimicked the albumin effect in serum, creating a stable environment and enhance accuracy.

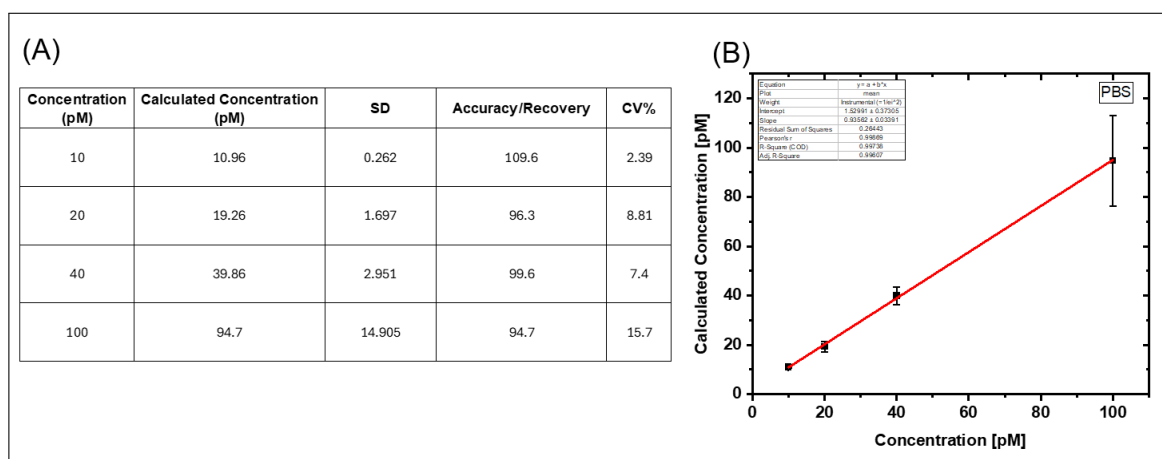


Figure 4.16 (A) The values for accuracy/recovery and coefficient of variation (CV%) in the concentration analysis of oligomer α -synuclein protein detection in PBS (B) A linearity plot illustrating the relationship between concentration and the calculated concentration based on the fitted model.

The concentrations of α -synuclein were spiked into the solution of the mimic serum; absorbance values were determined for each of the given concentrations. A linear regression analysis showed a strong correlation with a high coefficient of determination ($R^2 = 0.99$), indicating that the method is reliable. The recovery and coefficient of variation (CV) values were calculated to assess the accuracy and precision of the measurements. Recovery values fell within the acceptable range of 80–120% for all tested concentrations and gave specific Accuracy/recovery rates of 99.3% for 10 pM, 104.5% for 20 pM, 106.5% for 40 pM, and 96.06% for 100 pM. Repeatability was considered very good. CV values were 13.16% for 10 pM, 8.2% for 20 pM, 5.86 for 40 pM, and 13.38 for 100 pM. These findings show that the method retains both high repeatability and reproducibility, even at low concentration levels. The use of PBS + 1.5% BSA as a serum mimic environment effectively stabilized α -synuclein proteins, providing reliable results across the linear measurement range. These results are critical to method optimization and confirm that oligomeric α -synuclein can be reliably quantified in biological fluids, even at low concentrations. The high accuracy and strong linear correlation, with low variation, justify the applicability of the method for clinical and experimental purposes.

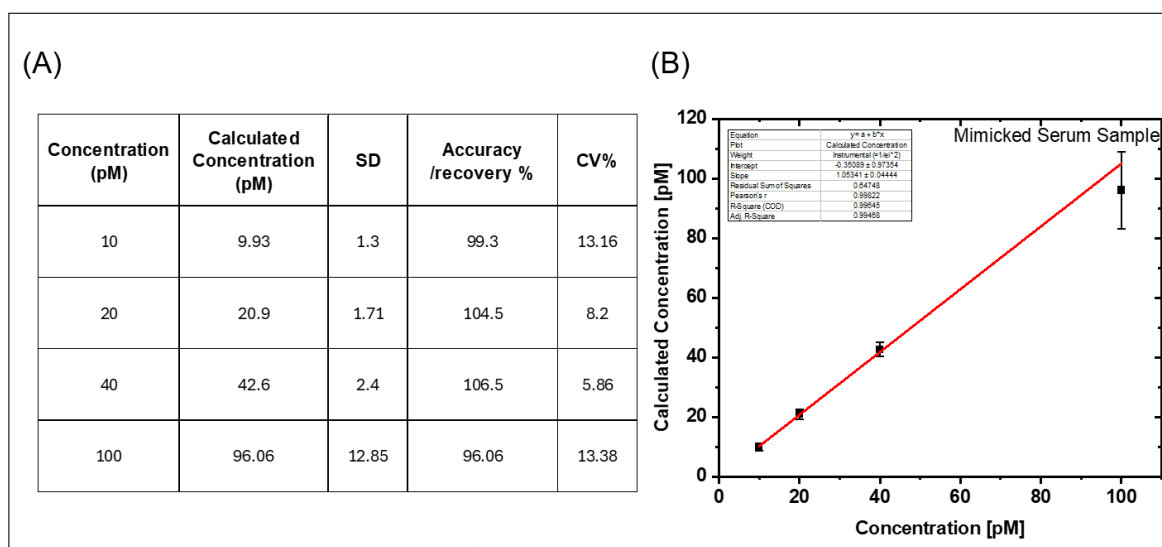


Figure 4.17 The values for accuracy/recovery and coefficient of variation (CV%) in the concentration analysis of oligomer α -synuclein protein detection in mimicked serum samples (B) A linearity plot illustrating the relationship between concentration and the calculated concentration based on the fitted model.

4.8 Selectivity Analysis for Fiber Optic Sensor Probe

The amino and hydroxyl groups in the CH polymer provide the immobilization of a wide range of biomolecules and form specific binding sites of biomolecules via these groups. The sample complex of cholesterol, hemoglobin, l-histidine, and monomer form of α -syn protein was chosen to test the oligomer form of alpha-synuclein plasmonic sensor selectivity. Hemoglobin is found in high concentrations in biological samples [115] cholesterol was chosen to evaluate its potential interactions with lipids on the CH surface and cholesterol, in particular, is found in blood and is a critical component to consider in ensuring accurate detection in biological samples [116] and L-histidine is a common amino acid found in the structure of proteins. It was chosen to see the interaction potential of the CH-coated sensor surface with amino acids [117]. In Figure 4.18, the response values of samples were obtained. The absorbance peak was lower than the oligomer alpha-synuclein (100%) peak value for cholesterol (8.33%), hemoglobin (10%), and L-Histidine (16.67%), and their response values were close. The selectivity % was calculated for alpha-synuclein monomers to be 58.33%. Monomeric and oligomeric forms of α -Syn have structural similarities but also significant differences. Disordered monomers (IDPs) are monomers have no specific three-dimensional structure and are

highly flexible. In contrast, oligomers are formed from the aggregation of monomers and usually have a more structured beta-sheet appearance and are generally more stable. Such structural characteristics could make the sensor respond similarly for both forms [118]. As indicated in Figure 4.18, the oligomer α -syn protein interacted strongly with the CH-coated fiber probe, and no significant shift was achieved in samples containing other interfering proteins. These cases all seem to indicate that the sensor can discriminate between oligomeric and monomeric forms of α -synuclein.

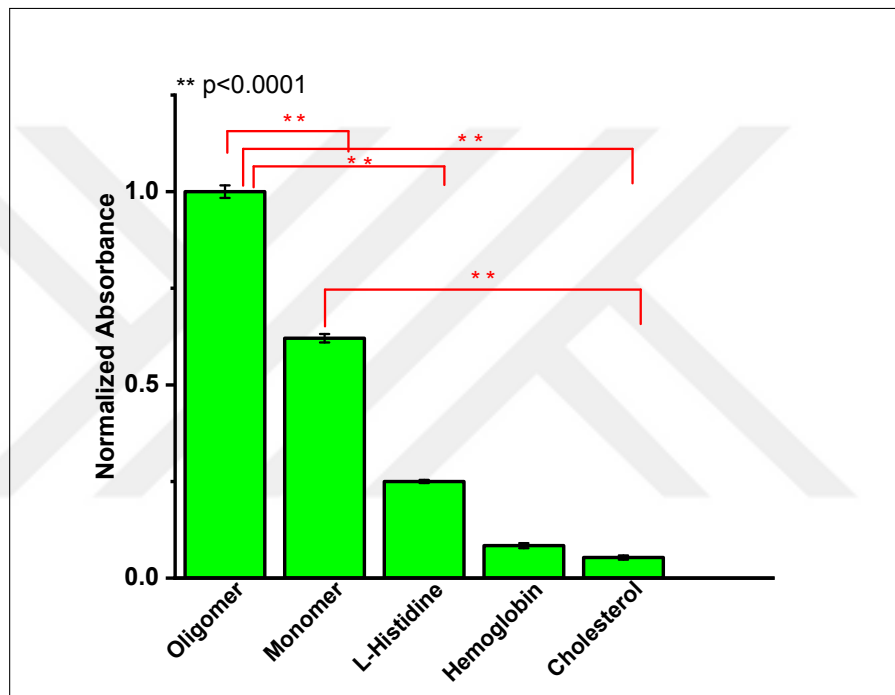


Figure 4.18 Selectivity test for oligomer form of α -syn. The response values of cholesterol, hemoglobin, L-histidine, monomer, and oligomer are separate. The error bars are standard deviations of three replicates. (** $p < 0.0001$)

In this study, CH was successfully used as the biorecognition element. However, the close values of monomer and oligomer indicate a need for enhanced selectivity. As shown in Table 4.1, different LOD values obtained for the detection of α -synuclein protein were reported. Future work could explore the use of specific antibodies or aptamers that are selective for oligomers together with CH to obtain a synergistic effect [119, 120]. Such biorecognition elements could enable more effective recognition of oligomers, thereby further improving system performance.

4.9 Response Time Analysis for Monomer and Oligomer α -Synuclein

The developed LSPR-based fiber optic biosensor response time was systematically evaluated for the detection of OA and MA. This is shown in Figure 4.19. The figure presents an absorbance spectrum over time (minutes) for both forms in monomeric (red line) and oligomeric (black line) α -synuclein. The results indicate distinct interaction kinetics for the two forms. The injection of an analyte containing monomeric α -synuclein results in a rapid response with a sharp increase in absorbance, reaching saturation within approximately 7 s. In contrast, the response to oligomeric α -synuclein shows a slower, more gradual increase, peaking at around 15 minutes, as illustrated in Figure 4.19. This response corresponds to previous LSPR-based sensors where rapid interaction of target analytes and sensor surfaces is one of the well-known advantages because localized plasmonic fields promote binding events [121].

Rapid response is very important in clinical applications since early detection of disease biomarkers is a priority. By contrast, the change in absorbance observed for the monomeric α -synuclein was significantly faster but less pronounced over the entire time frame, reaching saturation within 17 seconds. This distinction highlights the biosensor's higher specificity and sensitivity towards oligomeric α -synuclein, which exhibited a more gradual yet substantial increase, peaking at 15 minutes. In previous studies, where oligomeric proteins are shown to have higher binding affinities compared to their monomeric forms. This is attributed to their unique structural configurations and the presence of greater interaction sites, which facilitate stronger and more stable interactions [122, 123].

After the initial sharp increase in absorbance for oligomers, the signal reached a steady-state plateau, indicating sensor saturation. This stabilization suggests that the interaction between the oligomeric α -synuclein and the sensing region had reached equilibrium, a characteristic often observed in LSPR-based systems where molecular binding is both rapid and highly efficient. Stability of such sensors is crucial for

real-world applications, especially in point-of-care settings where reproducibility and consistency are non-negotiable. The 15-minute response time is a great improvement from conventional diagnostic methods such as ELISA, which may take hours to produce results [124]. The rapid response has an added advantage in the clinical arena given that timely detection of biomarkers could significantly change treatment outcomes.

This work also supports the results of earlier studies where the use of U-fiber optic biosensors improved performance due to their greater surface interaction area. The combined effect of U-shaped geometry and LSPR technology allows a highly sensitive response while maintaining low time for the response, further evidence of its capabilities of application in practical diagnostic purposes. The nearly 15-minute response time and the sensor's reliability over extended durations highlight its suitability for point-of-care and real-time monitoring applications. These findings mark an important milestone in the improvement of diagnostic approaches for Parkinson's disease, where early detection of toxic oligomeric species is essential for improving patient outcomes.

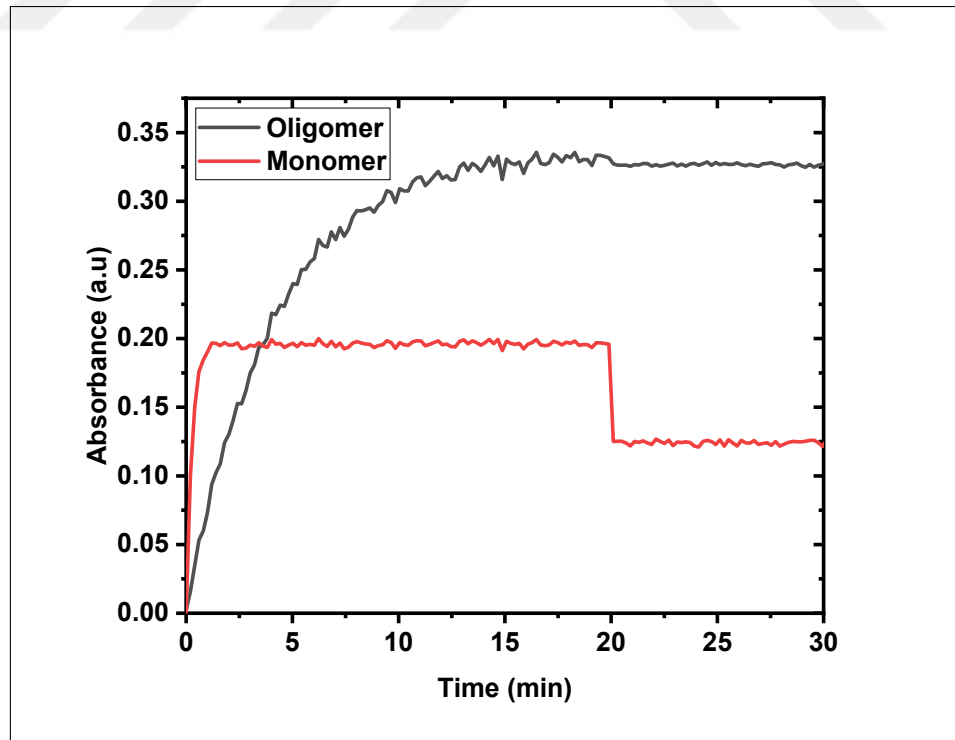


Figure 4.19 Response time analysis of monomer and oligomer α -synuclein: Absorbance spectrum for 100 pM α -synuclein oligomers after washing with PBS.

Table 4.1 Comparison of α -synuclein detection reported in the literature.

Sensor	Method	Limit of detection (LOD)	Reference
Electrochemical biosensor	EIS	55 pM	[120]
Electrochemical biosensor	EIS	1.13ng/mL	[125]
Electrochemical biosensor	CV	3.5×10^{-5} ng/mL	[126]
Electrochemical biosensor	SWV	0.03 fM	[127]
Electrochemical biosensor	CV	1 pM	[128]
Electrochemical biosensor	EIS	1.2 pM	[129]
Optical biosensor	Localized surface plasmon resonance (LSPR)	70 nM	[130]
Optical biosensor	Localized surface plasmon resonance (LSPR)	11 pM	This study

5. DISCUSSION AND FUTURE PERSPECTIVES

This research successfully highlights the U-shaped LSPR-based fiber optic biosensor designed to detect oligomer α -synuclein, a vital biomarker for Parkinson's disease, at very low concentrations. The sensor, utilizing MUA-capped gold nanorods (GNRs) and functionalized with chitosan (CH), demonstrated remarkable sensitivity and specificity. In this work, LOD and sensitivity value were found as 11 pM and 502.69 $\Delta\lambda/RIU$, respectively. This finding emphasizes the sensor's potential as a dependable tool for the early diagnosis of Parkinson's disease.

The performance of the proposed biosensor significantly surpasses conventional methods such as ELISA and spectroscopic techniques in terms of sensitivity, response time, and portability. While traditional methods require large, complex instrumentation and lengthy processing times, this LSPR-based fiber optic sensor offers a rapid, cost-effective, and field-deployable alternative. In this study, the use of MMF (multi-mode fiber) further enhances light coupling efficiency, ensuring high signal-to-noise ratios, which are critical for accurate detection. The U-shaped configuration of the fiber further enhances the interaction of light with the sensing surface, optimizing the LSPR signal and enabling the detection of low-concentration biomarkers.

The proposed biosensor demonstrates significant advantages for clinical applications, particularly in point-of-care settings. Its ability to detect oligomeric α -synuclein in the range of 10–100 pM aligns well with the concentrations observed in CSF, serum and plasma samples from Parkinson's disease patients [131]–[133]. Moreover, the fast detection time of 15 min for this sensor as a practical tool for real-time diagnostics. The LOD and time of detection reported in this study for oligomeric α -synuclein on optical sensors has yet to be published. Accordingly, this study helps to bridge an important gap in the literature.

Despite its promising results, the sensor faces some limitations that warrant further exploration. One key challenge is the selectivity between MA and OA. While the sensor showed higher affinity for oligomers, the selectivity was not absolute, with monomers producing a significant response. This limitation underscores the need for more specific biorecognition elements, such as aptamer conjugated chitosan, to enhance the sensor's ability to distinguish between structurally similar forms of α -synuclein.

Moreover, the lifetime stability of the sensor has not been examined due to fragility of the sensor. These are essential for the practical implementation of the former in clinical and field applications. This problem should be dealt with in future work using rigorous testing and more robust surface coatings or plastic optic fibers (POF).

This research establishes a basis for the development of LSPR-based biosensors for neurodegenerative disease diagnostics. Future research could focus on expanding the application of this sensor to detect other biomarkers associated with Parkinson's disease and related disorders. While lower sensitivities have been achieved when compared to the typical SPR assays the ability to operate without the utilization of an antibody makes this sensing platform unique [134]. Here, we report that our sensor can be used to distinguish between binding kinetics and response times of monomers and oligomers.

The simple and novel method was applied to create SAM. MUA-capped GNR was used in this study to improve the stability of GNRs and reduce functionalization time. Using this technique, the detection of oligomer form alpha synuclein protein was successfully achieved. This approach shows a new contribution to U-shaped fiber optical biosensor studies.

From a technological perspective, the potential for scaling this sensor to enable rapid, large-scale, and low-cost manufacturing holds promise. Integration of microfluidic systems with the sensor is also likely to lead to automated and integrated diagnostic platforms making it more appropriate for point-of-care applications.

This study introduced the U-shaped LSPR-based fiber optic biosensor which significantly improved the detection of oligomer α -synuclein. High sensitivity, specificity and quick response time demonstrate its potential for diagnosis of early Parkinson's disease. Future studies should also overcome the issues of selectivity and long-term stability to enhance its clinical relevance and ultimately improve patient outcomes and broaden the clinical utility of this novel biosensing technology.



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