

RESEARCH PAPER

## Tuning the Synthesis and Stabilization of Gold Nanorods for Enhanced LSPR Sensor Chip Performance

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### ABSTRACT

Plasmonic nanorods exhibit unique optical properties due to their ability to support localized surface plasmon resonances (LSPR). These nanorods are highly valued for applications in sensing, imaging, and medical therapies because of their tunable optical behavior and strong electromagnetic field enhancement. In this study, a seed-mediated approach was utilized to synthesize and purify gold nanorods (GNRs), with a focus on their integration in the LSPR sensor chips. The synthesis was begun with the preparation of small gold nanoparticles as seeds, followed by the growth of nanorods in a carefully controlled environment. UV-visible spectroscopy confirmed the formation of GNRs, revealing two plasmonic peaks, with the transverse peak at 523 nm and the longitudinal peak at 800 nm after 24 hours of aging. Further, the self-assembled monolayer of the GNRs on glass substrate achieved using a thiol-functionalized linker, 3-mercaptopropyltrimethoxysilane (MPTMS), and ionic strength modulation with NaCl. For optimal stabilization and alignment of the nanorods on the glass substrate, the ideal incubation time for the glass substrates in the colloidal gold nanoparticle solution was determined to be 24 hours. This duration was made the most effective configuration of the LSPR sensor chip, with improved stability and sensitivity for plasmonic applications.

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### INTRODUCTION

Plasmonic nanostructures exhibit unique optical properties, characterized by their optical cross-section, which makes them suitable for a wide range of applications, particularly in biosensing [1, 2]. One of the most significant optical phenomena in these structures is localized surface plasmon resonance (LSPR). The LSPR refers to the interaction between incident electromagnetic waves and plasmonic nanoparticles, leading to a collective oscillation of conduction electrons within the nanoparticles. This interaction makes

the distinct resonance peaks in the extinction spectra, which the wavelength and intensity are highly sensitive to the size, shape, and geometry of the nanoparticles, as well as to the dielectric environment surrounding them [3, 4]. These tunable properties make plasmonic nanostructures highly versatile for various applications in sensing technologies [5].

Recently, there is growing interest in non-spherical nanoparticles, such as gold nanorods (GNRs), nanoprisms, and nanourchins, due to their enhanced optical properties and expanded

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application potential [6, 7]. The GNRs are particularly attractive for biosensor applications due to their two distinct plasmonic peaks: one oriented along the long rod axis and the other perpendicular to it, referred to as the transverse LSPR (~520 nm) and longitudinal LSPR (~650–1350 nm) peaks, respectively. The longitudinal LSPR peak is especially sensitive, because it can be finely tuned across the visible and near-infrared (NIR) regions of the electromagnetic spectrum, enhancing its sensitivity in biological environments [8]. Additionally, their surface chemistry facilitates easy biological immobilization, further expanding their use in biosensing platforms.

Several methods have been developed for the synthesis of GNRs, including electrochemical deposition, template-assisted growth, photochemical processes, and seed-mediated growth [9, 10]. Although seedless growth of GNRs has been documented, the colloidal synthesis of GNRs fundamentally relies on the seed-mediated growth approach, first introduced by Murphy and El-Sayed [11, 12]. This method has been refined by various research groups [13, 14], to produce narrow size and shape distributions and high yields across various sizes and aspect ratios. The central principle of seed-mediated growth is the separation in space and time between the nucleation and growth of nanocrystals, which enables better control over the reaction and results in more uniform nanoparticles [15, 16].

Self-assembly monolayer is a highly effective method due to its simplicity, affordability, adaptability, and consistent results. As a result, it is considered one of the most promising techniques for arranging particles with specific properties into complex structures [17-19]. Currently, self-assembled GNRs are mainly applied in sensing biological or chemical substances and molecule identification via LSPR sensors. These sensors detect biochemical elements by monitoring shifts in plasmon wavelength peaks using UV-Vis or photoluminescence spectroscopy. In general, the LSPR sensors offer significant potential for advancing nanotechnology, with promising applications in sensing, and molecular detection [20, 21].

In this paper, we will explore the synthesis of GNRs using the seed-mediated growth method, detailing the two-step process for obtaining well-defined GNRs. Furthermore, we will investigate the assembly of GNRs on a glass substrate to

create an LSPR chip with varying fill factors, focusing on how different assembly densities affect the optical properties and performance of the nanorods in biosensing applications. The goal is to optimize the fabrication process and assembly of GNRs to enhance their potential for use in high-performance biosensors.

## MATERIAL AND METHOD

### Material

Gold (III) chloride trihydrate ( $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ ,  $\geq 99.9\%$ ), Cetyltrimethylammoniumbromide (CTAB,  $\geq 99\%$ ), Trisodium citrate dihydrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$ ,  $\geq 99\%$ ), Sodium Borohydride ( $\text{NaBH}_4$ ,  $\geq 99\%$ ), Silver Nitrate ( $\text{AgNO}_3$ ,  $\geq 99\%$ ), L-Ascorbic acid ( $\text{C}_6\text{H}_8\text{O}_6$ ,  $\geq 99\%$ ), 3-mercaptopropyltrimethoxysilane (MPTMS,  $\geq 97\%$ ), Hydrochloric acid (HCl, 37%), Ethanol (99%), Methanol (MeOH, 98%) and Sulfuric acid ( $\text{H}_2\text{SO}_4$ , 98%) were purchased from Merck. Ultrapure DI water with a resistivity of 18.2 M $\Omega$  cm was used.

### Synthesis of gold nanorods

#### Seed process

In the first stage, very small Gold NPs (approximately 5 nm) with a cubic structure, known as seed solution, were synthesized. Initially, 3.5 ml of CTAB was dissolved in DI water at a concentration of 0.1 M by sonicating until the solution became completely colorless and free of its gel-like state. The surfactant must dissolve through sonication, as heating can adversely affect the seed growth process [22]. Then, 22  $\mu\text{l}$  of  $\text{HAuCl}_4$  at a concentration of 0.04 M, which had been prepared in advance, was added all at once to the vigorously stirring surfactant solution. The solution was placed on a heater-stirrer at 25°C and stirred at high speed until it turned a uniform yellow color.

Before starting the synthesis,  $\text{NaBH}_4$  solution was prepared by sonicating  $\text{NaBH}_4$  in cold DI water and stored in an ice bath in a refrigerator. Then, 30  $\mu\text{l}$  of the 0.1 M  $\text{NaBH}_4$  solution, which had been kept in the refrigerator to bubble, was added all at once to the seed solution. Then the container was sealed and shaken vigorously for 2 minutes. Due to the vigorous shaking, the solution became foamy, which was attributed to the use of the CTAB surfactant. This step was very sensitive and critical. During the shaking, the solution's color was changed to light brown, indicating the formation of the seed NPs. The color of the seed

solution was highly depended on the quality of the synthesis. Finally, the seed solution was placed in a dark environment at room temperature for 2 hours to age for the growth step.

#### Growth process

At this stage, known as the growth step, 8.5 ml of surfactant solution (CTAB) at a concentration of 0.1 M was prepared similarly to the seed step and placed on a heater at 30°C. While the solution was gently stirred, 71.5 µl of AgNO<sub>3</sub> solution at a concentration of 0.01 M was slowly added to the system. This solution must be freshly prepared at the time of use. Next, 107 µl of HAuCl<sub>4</sub> (0.04 M) was slowly added, causing a change in the solution's color from colorless to yellow. A few minutes after thorough mixing, 73 µl of Ascorbic acid solution (0.1 M) - a weak reducing agent - was slowly added, resulting in the solution gradually becoming colorless. It is critical at this stage that the solution becomes completely colorless upon the complete addition of Ascorbic acid.

To adjust the pH of the solution, 50 µl of a diluted 1 M HCl solution was slowly added, changing the solution's pH from 6 to 4. The growth solution was then readied for the addition of the seed solution. Subsequently, 30 µl of the seed solution was slowly added to the growth solution, and within 2 to 3 minutes, the solution's color was changed to purple and then gradually to gray or dark violet, indicating the formation of GNRs. The purple, violet, and gray colors were signified different sizes of nanorods formation. Controlling the amount of AgNO<sub>3</sub> is crucial for regulating the size and

geometry of the nanorods, as it directly influences the rod-like growth of the seed nanoparticles. The ratio of AgNO<sub>3</sub> and Ascorbic acid to HAuCl<sub>4</sub> was optimized to produce uniform gold nanorods with a longitudinal plasmon peak in the near-infrared region. A 6:1 ratio of HAuCl<sub>4</sub> to AgNO<sub>3</sub> and a 1:1.7 ratio of HAuCl<sub>4</sub> to Ascorbic acid was found to result in optimal growth, yielding well-defined nanorods with the desired optical properties. Finally, the nanorod solution was incubated at 30°C overnight to complete the growth phase. Fig. 1 shows the growth and seed solutions, with the gray color indicating the presence of Gold NRs.

#### Purification of Gold Nanorods

After the growth phase of GNRs was completed, the solution had to be purified to remove excess surfactant (high concentrations of CTAB were used in both growth and seed steps), spherical NPs, and unreacted Au ions. The solution was centrifuged 4 times at 1100 rpm for 10 minutes each time. Increasing the centrifugation time or speed can cause irreversible agglomeration of the nanoparticles. The supernatant was discarded, and an equal volume of DI water was added. To prevent possible agglomeration and ensure uniform distribution of the GNRs, it was sonicated for 10 minutes after each centrifugation. The transmission electron microscopy (TEM) images of colloidal GNRs are depicted in Fig. 2a and 2b. As seen in the images, the nanorods were not monodisperse but polydisperse. The average sizes of the nanorods were calculated to be 50 nm in length and 15 nm in width, according to Fig. 2c, an

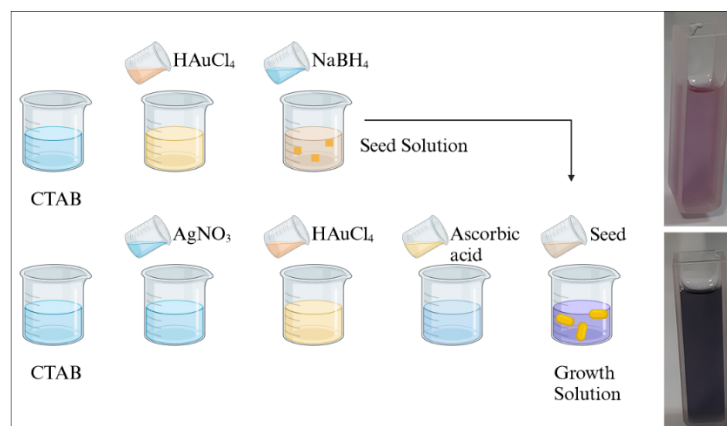


Fig. 1. Scheme of synthesis of GNRs with seed-mediated growth method, the Seed solution with pink color is shown in the up, also the Growth solution with gray color is shown in the down of the picture.

aspect ratio was 3.33.

**RESULT AND DISCUSSION**

*Localized Surface Plasmon Resonance of Gold Nanorods*

After synthesis and purification, GNRs are stored in colloidal form in a refrigerator for later use in assembling onto the glass substrate. UV-visible spectroscopy (OPTC Company, Iran) is employed to investigate the plasmonic oscillations of the GNRs. The extinction spectrum of the nanorods is measured immediately after their synthesis, after 12 and 24 hours (h) as shown

in Fig. 3b. The nanorods exhibit two distinct plasmonic resonance peaks, the high energy peak corresponds to plasmonic oscillations along the width of the rods (transverse peak), while the low energy corresponds to plasmonic oscillations along the length of the rods (longitudinal peak). The presence of these two peaks in the extinction spectrum confirms the formation of GNRs. With increasing aging time, the low energy peaks are shifted to higher wavelengths due to the growth of nanorods to longer lengths. The transverse peaks were consistently observed at 523 nm across all spectra, while the longitudinal peaks appeared at

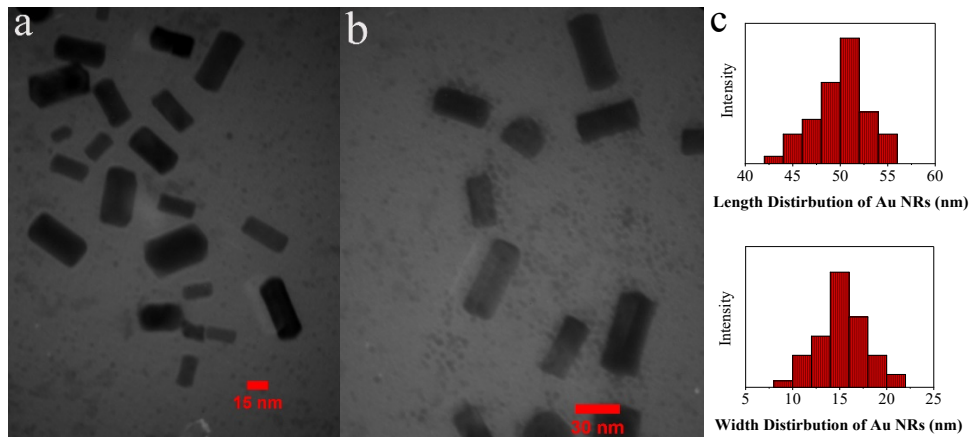


Fig. 2. The TEM Images of GNRs, a) in 15 nm scale b) in 30 nm scale, c) The average sizes of the nanorods were calculated. The length distribution, width distribution, and aspect ratio were 50 nm, 15 nm, and 3.33, respectively.

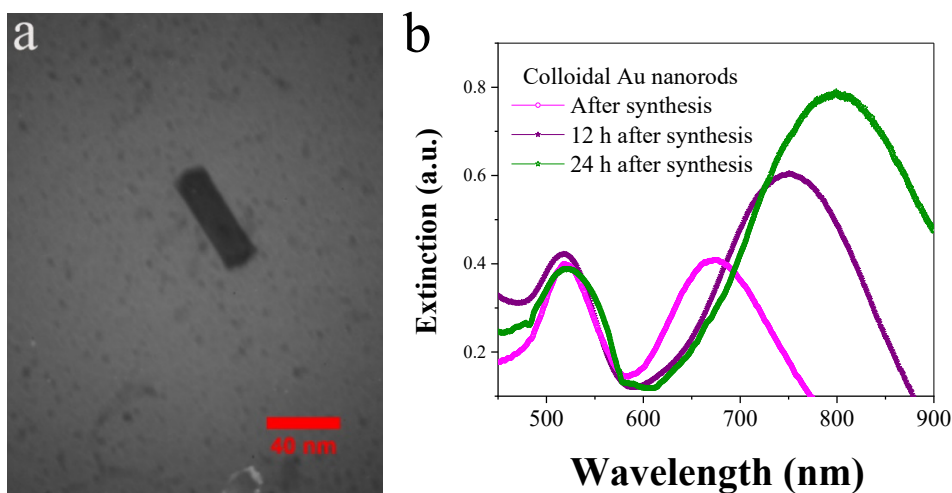


Fig. 3. The TEM images of a single Gold nanorod, the extinction spectrum of colloidal GNRs exhibiting LSPR, with two plasmonic resonance peaks: the transverse peak at 523 nm and the longitudinal peaks, immediately after synthesis, after 12 h, and 24 h at 670, 753, and 800 nm, respectively.

670, 753, and 800 nm. The TEM image of a single colloidal GNR after 24 h of aging is shown in Fig 3a, during which the nanorods matured into their long rod geometry, suitable to fabricate the LSPR sensor chip [23].

*Stabilization of Gold Nanorods on a Glass Substrate*

GNRs were synthesized using a cationic surfactant (CTAB). Therefore, a suitable linker is required to attach the GNRs, stabilized by the cationic surfactant, to the glass surface. Glass is frequently used as a host material because of its rigidity, affordability, and the abundance

of exposed surface hydroxyl groups (OH) [24]. Additionally, its optical transparency is a significant advantage. Before binding the GNRs suspended in colloidal solutions, the glass surface must be prepared by treating it with sulfuric acid, followed by a rinse with Methanol. The chosen linker, 3-mercaptopropyltrimethoxysilane (MPTMS), contains a thiol functional group for binding to the GNRs [25]. During the synthesis of GNRs, a high concentration of the surfactant CTAB is used, and after centrifugation, a certain amount of CTAB has remained to prevent aggregation of the nanorods. Attempts to stabilize the nanorods on a glass

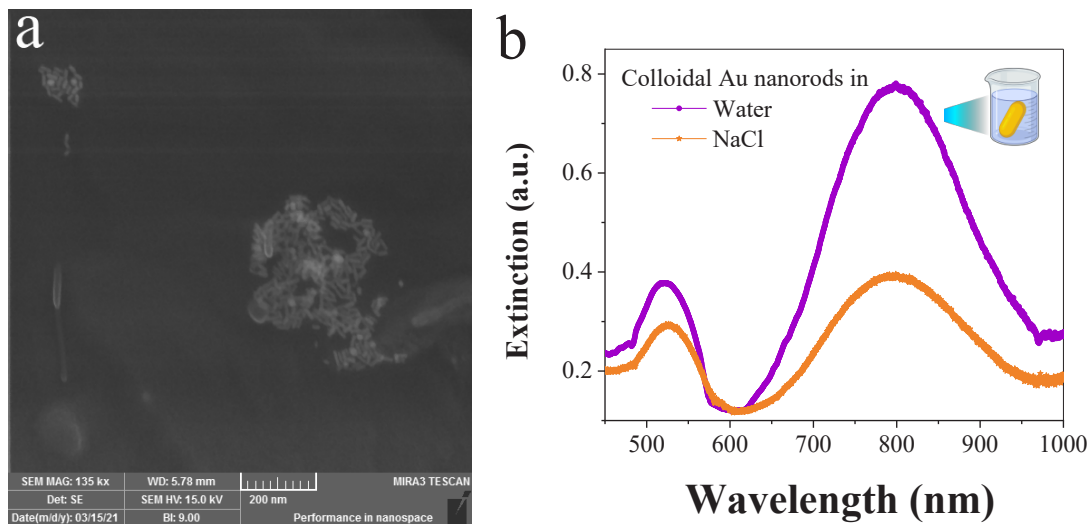


Fig. 4. a) The SEM image of GNRs in NaCl solution, b) The extinction spectra of GNRs, colloidal in water and NaCl solution.

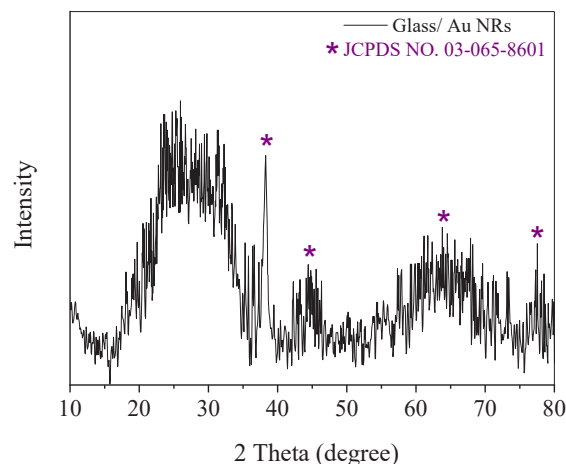


Fig. 5. X-ray diffraction pattern of GNRs assembled on the glass substrate.

substrate using the linker resulted in poor adhesion of the nanorods to the glass surface, with only a few attaching and producing weak plasmonic peaks. To address this, negatively charged ions were introduced to modify the nanorod micelles, achieved by using a NaCl solution [26]. As a result, after centrifuging the GNRs and removing the excess surfactant, the nanorods were dissolved in a 50 M NaCl solution. The ionic strength was controllable by adding salt to GNRs solution with minimally excess surfactant. The extinction spectra of GNRs, colloidal in water and NaCl are depicted in Fig. 4b. At 50 M concentration, only a decrease in extinction at constant transverse and longitudinal peak wavelengths is detected throughout the incubation, suggesting that mild aggregation gradually takes place without distortion of GNRs, while the scanning electron microscopy (SEM) is shown GNRs in NaCl solution with rod geometry in Fig. 4a.

Pellas et al. employed a method of alternating treatments with polycations and polyanions on GNRs-coated glass slides to build a layer-by-

layer structure of increasing thickness, while simultaneously monitoring the position of the LSPR band [27]. Peixoto et al. presented a quick and straightforward method for a surface enhanced fluorescence based immunoassay utilizing GNRs. The sandwich immunoassay was created by combining (i) GNRs in suspension, modified with BSA and the reporter molecule, with (ii) GNRs adsorbed on a glass slide (GNRs-chip) linked to anti-BSA [28]. Wang et al. showed that minor aggregation, triggered by low ionic strength (NaCl), can promote nanorod assembly, leading to the formation of a dense and evenly distributed surface monolayer [29]. The stabilization of GNRs on the glass substrate as an LSPR chip via the self-assembled monolayer method is done. First, to clean the glass substrates, they are sonicated for 5 minutes in acetone and then for another 5 minutes in ethanol. Next, the glass substrates are thoroughly dried with nitrogen gas and incubated overnight in a solution of MPTMS linker, which is diluted to a concentration of 0.5% with 96% ethanol. To remove unbound linkers from the glass

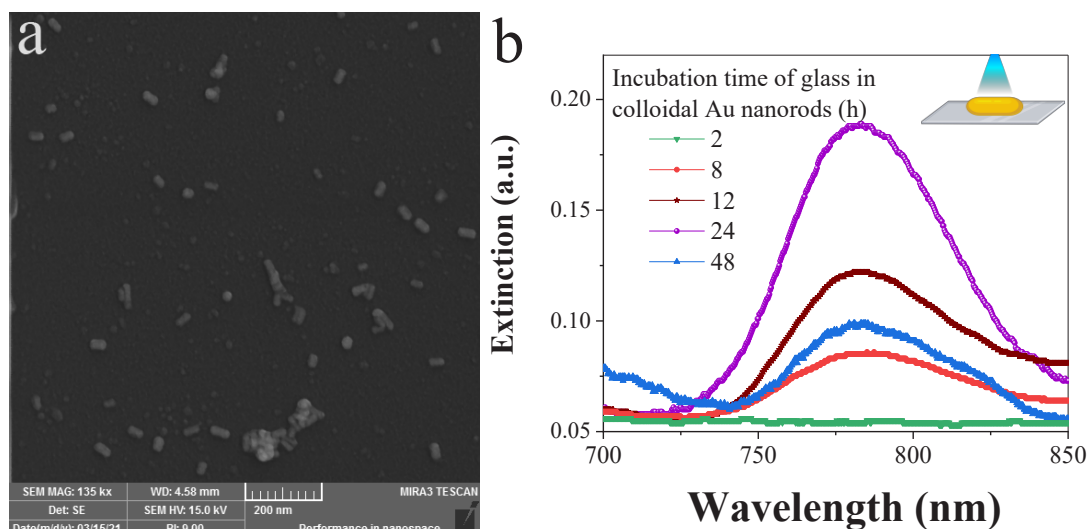


Fig. 6. a) The SEM images of GNRs stabilized on a glass substrate as an LSPR chip after 24 h incubation time in the colloidal GNRs with NaCl as the solvent, b) Extinction spectra of GNRs stabilized on a glass substrate with different incubation time: 2, 8, 12, 24, 48 h.

Table 1. The extinction wavelengths, and intensity of longitudinal plasmon peak of LSPR chip for different incubation times: 2, 8, 12, 24, and 48 h.

	2h	8h	12h	24h	48h
Wavelength (nm)	785	730	783	783	782
Intensity of extinction (a. u.)	0.038	0.099	0.188	0.273	0.122

surface, the substrates are sequentially sonicated for 10 minutes in ethanol and then twice for 10 minutes in DI water. To improve the temperature of the substrate and enhance the attachment of the nanorods to the linker, the substrates are placed in an oven at 120°C for 2 hours. The XRD pattern of GNRs assembled on the glass substrate is shown in Fig. 5, the diffraction peaks correspond to the face-centered cubic (fcc) crystalline planes (111), (200), (220), and (311), aligning with the reference data from JCPDS No. 03-065-8601 for Au metal. The separation between the GNRs on the glass substrate surface is evidenced by the low intensity observed in the XRD pattern.

Then, the MPTMS-functionalized substrates are immediately immersed in GNRs solution for 2, 8, 12, 24, and 48 hours. To further investigate of LSPR peaks of the LSPR chip, the extinction spectra of glass substrate with stabilized GNRs are demonstrated in Fig. 6b. In addition, the presence of a single plasmon peak at 783 nm, corresponding to the longitudinal peak, is confirmed that the nanorods are aligned along their lengths. This alignment is further supported by the SEM image taken after 24 hours of incubation in colloidal GNRs (Fig. 6a), showing the nanorods assembled with a higher fill factor and greater intensity compared to other incubation times.

According to Table 1, the extinction spectra of the LSPR chip indicate that the number of nanorods stabilized on the substrate was negligible for the 2 h of incubation, and no plasmonic peaks were observed. The lack of a significant amount of nanorods after 48 h of incubation is attributed to the fact that after 12 h, the nanorods had sedimented, leaving very few particles suspended in the solution. Additionally, the weak attachment of the nanorods to the glass surface was found to be another contributing factor, leading to their detachment and sedimentation as the incubation time increased. It is worth noting that the optimal conditions for both the GNRs and the LSPR sensor chip were achieved with 24 h of aging time for the nanorods and 24 h of incubation time for the chip, respectively. This incubation time was found to produce the most effective LSPR chip configuration, ensuring enhanced stability and sensitivity for LSPR applications.

## CONCLUSION

The seed-mediated growth method was employed to synthesize colloidal GNRs to create

the LSPR sensor chips. The GNRs were found to be polydisperse, with an average size of 50 nm in length and 15 nm in width, yielding an aspect ratio of 3.33. The UV-visible spectroscopy confirmed the presence of two distinct plasmonic resonance peaks, corresponding to transverse and longitudinal plasmonic oscillations of the GNRs. As the aging time of GNRs increased from 0 to 24 h, the longitudinal plasmonic peaks shifted to higher wavelengths, indicating the growth of the nanorods. At the optimal aging time of 24 h, the longitudinal peak was measured at 800 nm. Stabilization of the GNRs on glass substrates proved challenging, requiring the use of a thiol-functionalized linker (MPTMS) and careful manipulation of ionic strength via NaCl. After testing various incubation times of glass substrate inside colloidal GNRs, it was determined that 24 h of incubation time was optimal. This duration allowed the nanorods to achieve better assembly, alignment, and higher fill factor, leading to the most effective LSPR sensor chip configuration. In conclusion, the 24 h of incubation time provided the best conditions for the preparation of stable and sensitive LSPR sensor chips, enabling enhanced performance for plasmonic sensing.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding this article.

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