Sensitivity Analyses of Cu/Chitosan and Ag/Chitosan Based SPR Biosensor for Glucose Detection

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Abstract. This study investigates the sensitivity performance between hybrid thin films of Copper (Cu)/Chitosan and silver (Ag)/Chitosan for glucose biosensing applications. Ag and Cu with refractive indices of $n=0.1351+3.9853k$ and $n=0.2388+3.4156k$ are coated onto the flat surface of hemispherical prism prior 10nm thickness of chitosan ($n=1.54+0.015k$). The thicknesses of metal are varied between 35nm until 49nm. To generate SPR, a red laser of 633nm $p$-polarized light is incident onto the Cu/Chitosan and Ag/Chitosan coated prism. Light incident angles are varied from 40° to 60° via the angular interrogation technique. Glucose solution with a concentration of 70mg/dl and 235mg/dl (1.38 RIU and 1.53 RIU, respectively) are flown along with the flow cell during SPR to investigate the sensing ability of the proposed sensors. The relationship between hybrid thin film thicknesses and the value of minimum reflectance shows a polynomial pattern as the thicknesses increased. Based on $Q^2$ analysis, the deployment of Ag/Chitosan results in 18.51% poorer stability performance of $R_{min}$ value than Cu/Chitosan SPR sensor. The Cu/Chitosan SPR sensor at total thicknesses within the range from 46nm to 49nm and from 53nm to 56nm exhibits 67% better potential than Ag/Chitosan due to its sensitivity and selectivity in differentiating dissimilar concentration of glucose with a maximum sensitivity of 6°/RIU. We believe the utilization of Cu/Chitosan as plasmonic sensing material offers a low-cost sensor that easy to handle, cheap, miniaturized and excellent sensitivity.

1. Introduction
Glucose control daily monitoring is the most important indicator in detecting diabetes symptom by monitoring its concentration. The most common method of glucose monitoring includes collection of a mixture of capillary blood and by finger pricking [1]. However, finger-sticking is inconvenient because most of the patients feel uncomfortable and painful, which can result in less frequent testing and consequently a poorer control of blood glucose levels. Most diabetic patients struggle to regulate their blood glucose level (BGL) in the desired range. The normal physiological range, typically defined as 72-180 mg/dl. Hypoglycaemia occurs when the BGL is too low (<70 mg/dl) which can lead to death. Hyperglycaemia happens when the BGL is higher than 180 mg/dl and can cause to serious long-term complications, including kidney damage [2].

Recently, researchers show their interest in developing sensing device for blood glucose detection. A non-invasive near-infrared (NIR) technique shows a promising approach for blood glucose measurement detection [3-7]. Combination of three different types of techniques such as electromagnetic, acoustic speed and NIR spectroscopy can minimize the low performance of single-technique approaches [8]. Other than that, Raman spectroscopy also portrays an excellent technique for glucose detection [9]. With the proposed Hematocrit compensation mechanism, an electrochemical
biosensor shows an excellent candidate to detect diabetic disease [10]. Ahmad et al. (2017) have grown vertically-aligned ZnO nanorods (NRs) on fluorine-doped tin oxide (FTO) electrodes and decorated with CuO to achieve a high-performance non-enzymatic glucose sensor [11].

Surface plasmon resonance (SPR) is one of the popular optical phenomenon created by surface plasmon polaritons (SPP) which has been exploited for sensing application based on detection of the refractive index of the surrounding medium. It can be produced by applying light onto noble metal such as gold and silver. The SPP excitations occur at the dielectric-metal interface with the presence of glass and metal prisms. The positive dielectric constant of glass and negative dielectric constant of Au cause vertical oscillations resulting in the SPP excitation. The frequency of SPP excitation, $\omega_{spp}$ is always smaller than the optical frequency, $\omega_{opt}$. When light propagates from air directly incident onto the metal at $\omega = ck$, the SPP excitation does not exist considering the wavevectors mismatching. By using indexed prism $n_i$, the wavevectors between the prism, $k_i$ and SPP, $k_{spp}$ able to be matched. Higher refractive index prisms allow longer wavevectors to be generated at the dielectric-metal interface. The wavevector of the prism, $k_i$, propagating on the dielectric-metal interface, is calculated using Equation 1:

$$k_i = k_n \sin \theta$$  \hspace{1cm} (1)

By setting the value of $k_n$ (free space wavevector) and incident angle $\theta$, it is apparent that the refractive index value is directly proportional to the wavevector on the prism surface.

Few techniques have been introduced to create SPR such as fiber optics-based and prism coupling [12, 13]. Until today, gold is the most favorable materials due to its stability and prone to oxidization [14, 15]. Nonetheless, this material is expensive. To overcome this issue, many researchers have actively studied the potential of low cost and highly sensitivity alternative materials. Tabassum et al. (2016) investigated the influence of different high-index oxide overlayers on the performance of a fiber optic SPR sensor coated with bimetallic layer of aluminum (Al)/copper (Cu) [16]. They found that the usage of Al/Cu/TeO$_2$ layers over unclad core shows the best sensitivity for the detection of analyte with various refractive indices. Other than gold, silver (Ag) is a suitable candidate to excite SPR [17-19]. The application of Ag coated SPR sensor covers many applications such as for environmental safety, food security and biomedical applications [20-22]. The introduction of hybrid thin films is also found able to enhance the sensitivity of SPR sensor which are Ag/GO, Au/GO, Au thin films/Au nanoparticles, CNT/Cu and Ag/Chitosan/GO [23, 25]. The usage of chitosan in the optical sensor due to its conductivity property recently indicates the significance of this material for sensing applications. It has been proven that the deployment of metal with an additional chitosan layer will increase the sensitivity of the sensor due to the enhancement of SPP excitation [26]. Because of its sorbent for heavy metal ions, chitosan has been widely used for environmental sensing application to detect heavy metal ions in water [27-28].

Lately, the SPR sensor also has been utilized for blood glucose detection. Li et al. (2017) developed an optical surface plasmon resonance (SPR) sensor modified by glucose/galactose-binding (GGB) glucose detection [29]. Menon et al. (2019) introduced an Au/Cr for glucose sensing device via Krestchmann configuration [30]. The glucose monitoring also was successfully performed by using a side-polished fiber SPR sensor modified by graphene and borate polymer with temperature self-compensation in situ by long-period fiber grating (LPFG) [31]. A D-shaped dual-core externally Au metal-coated PCF inducing SPR sensor shows remarkable amplitude sensitivity for the detection of different environmental samples, including glucose [32]. This study is carried out to investigate the potential of silver (Ag)/Chitosan and Copper (Cu)/Chitosan as a plasmonic sensing material for glucose detection with various concentration. The concentration of glucose is matched with a normal person and hyperglycaemia patient, which are 70mg/dl and 235mg/dl, respectively. An angular interrogation technique using Kretschmann configuration is used to generate SPR. Thicknesses of metal thin films are varied from 35nm until 49nm to determine the suitable thicknesses of Ag and Cu. The proposed SPR sensor is then exposed to the water and glucose solution in order to evaluate the sensitivity of the sensor. The output of this work exhibits a bright potential of low cost Cu/Chitosan based SPR sensor for diabetics monitoring.
2. Materials and Methods

This study was performed theoretically by using Winspall 3.02 simulation software based on Fresnel equation [33-35]. A hemispherical prism was used to couple light from the p-polarized 633nm laser source, as illustrated in Fig. 1. The incident angle was controlled by rotating the angle from 40° until 60° with an increment of 0.30° per reading. As \( k = k_{spp} \), the resonance will achieve resulted in minimum light reflectance. At this condition, almost 100% of light is converted into SPP resulted in the SPR phenomenon. Two types of hybrid thin films were deployed in this study, namely silver (Ag)/ Chitosan and Copper (Cu)/ Chitosan. Both films were coated onto the flat surface of the hemispherical prism. Thicknesses of metal thin films were varied between 35nm to 49nm, meanwhile chitosan was kept constant at 10nm. The value of reflected light intensity was recorded based on the value of minimum reflectance, \( R_{min} \). For biosensing application, water and glucose which acted as analytes flowed along the flow cell simultaneously with the increment of incident angle. In this work, water (1.33 RIU) and glucose with different concentration, which are 70mg/dl (1.38 RIU) and 235mg/dl (1.53 RIU) were used as sensing media. It is noteworthy to highlight that the glucose concentration values were similar to human blood concentration of normal (70mg/dl) and diabetic person (235mg/dl). Analyses on the effect of Ag/Chitosan and Cu/Chitosan thicknesses on SPR angle shifting, Q-factor and sensitivity were investigated. Table 1 lists the parameter and RI value of materials used in this study.

![Figure 1: Illustration of Ag@Cu/Chitosan SPR sensor for glucose detection](image_url)

**Table 1:** List of materials and refractive index values

| Materials      | Refractive index | Thickness, t (nm) |
|----------------|-----------------|------------------|
| Glass prism    | 1.50            | -                |
| Ag             | 0.1351+3.9853k  | 35-49nm          |
| Cu             | 0.2388+3.4156k  | 35-49nm          |
| Chitosan       | 1.54+0.015 [36] | 10nm             |
| Glucose        | 1.38 (concentration=70mg/dl) [37] | - |
| Glucose        | 1.53 (concentration=235mg/dl) [37] | - |
| Water          | 1.33            | -                |
3. Results and Discussions

Fig. 2 illustrates the SPR curves as light was incident onto the Ag/Chitosan coated prism that had been exposed to the sensing media such as water, glucose with 1.38 RIU and 1.53 RIU. The Ag thin film thicknesses were varied from 35nm to 49nm. Meanwhile, the chitosan layer remained constant at 10nm. When the sensor exposed to the water, the SPR angle was obtained approximately at 44.97° with values of $R_{\text{min}}$ were between 0.0104 a.u and 0.1518 a.u as the Ag thicknesses increased (Fig. 2(a)). The SPR angles experienced blue-shifted about 2.20% once the sensing medium was replaced to glucose with RI=1.38 as illustrated in Fig. 2(b). Under this condition, the angles were found to be located at 45.28° as Ag thicknesses were set from 35nm until 39nm. From $t=40$nm until $t=49$nm, the angles were shifted to 44.97°. Almost similar results were recorded as glucose with 1.53 RIU took place in which the angle was blue-shifted to 44.97° started from $t=43$nm to $t=49$nm (Fig. 2(c)).

![Figure 2: Variation of SPR curves as Ag were varied between 35nm and 49nm, meanwhile the Chitosan layer was kept constant at 10nm using different sensing media (a) water (b) glucose with a concentration of 70 mg/dl (1.38 RIU) (c) glucose with a concentration of 235 mg/dl (1.53 RIU)](image)

Fig. 3 shows the SPR curves of Cu/Chitosan SPR sensor. Apparently, the width of SPR curves were wider than Ag/Chitosan based SPR sensor in which gave the first impression of a good sensitivity sensor [38, 39]. Various thicknesses of Cu/Chitosan films exhibited an obvious different reaction of angle shifting in which the SPR angles were blue-shifted from 46.48° to 45.88° the moment water was used as sensing medium (Figure 3(a)). In contrast, the Ag/Chitosan did not experience any peak shifting once it was exposed to water. The $R_{\text{min}}$ values were obtained between 0.0011 a.u and 0.0707 a.u. As the sensing medium replaced with different RI of glucose solution (1.38 RIU and 1.53 RIU), about 2.00% of angle shifting occurred for both solutions with the increment of film’s thicknesses from 35nm to 49nm (Figure 3(b) and 3(c)). The angle shifting validates the ability of both Ag/Chitosan and Cu/Chitosan sensors in detecting the presence of glucose with various concentration.
Figure 3: Variation of SPR curves as thicknesses of Cu were varied between 35nm and 49nm, meanwhile the Chitosan layer was kept constant at 10nm using different sensing media (a) water (b) glucose with a concentration of 70 mg/dl (1.38 RIU) (c) glucose with a concentration of 235 mg/dl (1.53 RIU). The relationship between hybrid thin film thicknesses and the value of minimum reflectance exhibits a polynomial pattern, as appear in Fig. 4. In comparison, $R_{min}$ values for Ag/Chitosan were greater than Cu/Chitosan. To study the performance stability of both sensors, $R^2$ analyses were performed by monitoring the $R_{min}$'s value. Note that, the closer the value to 1 ($R^2 \approx 1$), the better the consistency of the output in fitting their values with the polynomial lines. Slightly unstable results by Ag/Chitosan apparently noticed as the sensing media were replaced to glucose with refractive indices of 1.38 RIU and 1.53 RIU with $R^2$ values were obtained as 0.9499 a.u and 0.8411 a.u. (Fig. 4(a)). The Cu/Chitosan SPR sensor exhibited better stability as it was exposed to various sensing media in which the value of $R^2$ was almost 1 (Fig. 4(b)). Table 2 lists the $R^2$ values for various sensing media detected by Ag/Chitosan and Cu/Chitosan SPR sensors. Note that the Ag/Chitosan shows poor stability performance during glucose detection (1.53 RIU) with a percentage of stability that was 18.51% less than Cu/Chitosan.
Figure 4: Effect of metal thin film thicknesses, $t$ (nm) on the value of minimum reflectance, $R_{min}$

Table 2: $R^2$ values for various types of sensing medium using Ag/Chitosan and Cu/Chitosan thin films

| Sensing medium     | Ag/Chitosan | Cu/Chitosan |
|--------------------|-------------|-------------|
| Water              | 0.9994      | 0.9881      |
| Glucose (RI=1.38)  | 0.9449      | 0.9800      |
| Glucose (RI=1.53)  | 0.8411      | 0.9968      |

Fig. 5 displays the Q-factor values of Ag/Chitosan and Cu/Chitosan thin films during SPR. Note that the Q-factor represents the amount of light that was successfully converted into SPP. The larger the Q-factor value, the greater the intensity of SPR. In general, it was found that the Ag/Chitosan (Fig. 5(a)) thin films produced a larger Q-factor than Cu/Chitosan (Fig. 5(b)). The greatest Q-factor was resulted as the SPR sensor was exposed to the glucose (1.53 RIU) in which about 88% of SPP was successfully excited as the thickness of Ag was set at 46nm. Meanwhile, as the concentration of glucose reduced to 70mg/dl with 1.38 RIU, the maximum excitation of SPP, about 85% was excited as SPP at $t=47$nm. The lowest Q-factor value was resulted when $t=35$nm for both sensing media with the range of SPP excitations were within 74% until 77%. As depicted in Fig. 5(b), the Q-factor decreased about 3.17% with the usage of Cu/Chitosan thin films in comparison with Ag/Chitosan. As this sensor exposed to water, glucose with 1.53 RIU and 1.38 RIU; maximum Q-factor values were obtained at different thicknesses such as 42nm, 43nm and 45nm with percentage of SPP excitations about 85.21%, 85.24% and 84.71% respectively. Table 3 indicates value of maximum Q-factor at certain metal’s thicknesses as Ag/Chitosan and Cu/Chitosan were exposed to various types of sensing media.

Figure 5: Variation of Q-factor values as thin film thicknesses increased from 35nm to 49nm
Table 3: Maximum Q-factor at certain metal’s thickness as Ag/Chitosan and Cu/Chitosan were exposed to various types of sensing media

| Sensing medium | Ag/Chitosan | Cu/Chitosan |
|----------------|-------------|-------------|
|                | Maximum Q-factor | Thickness of Ag, t (nm) | Maximum Q-factor | Thickness of Cu, t (nm) |
| Water          | 0.8759       | 43          | 0.8521       | 42          |
| Glucose (RI=1.38) | 0.8469    | 47          | 0.8471       | 45          |
| Glucose (RI=1.53) | 0.8769    | 46          | 0.8524       | 43          |

Fig. 6 displays the shifting of SPR angle as different sensing media were introduced on top of the sensors. This analysis is very important to determine sensor’s sensitivity. According to Fig. 6(a), the SPR angles were shifted from 44.97° to 45.28° with percentage difference of 0.67% as the Ag/Chitosan SPR sensor with thicknesses within 35nm to 42nm was exposed from water to glucose. Nonetheless, at those thicknesses, the sensor unable to distinguish the presence of glucose with different concentration by considering no angle shifting occurred. The Ag/Chitosan sensor demonstrated its sensing ability to detect different concentration of glucose as the Ag thicknesses were set between 40nm to 43nm. Beyond 43nm, the sensor unable to distinguish both types of glucose solution. This situation explains the disadvantage of Ag/Chitosan thin film for glucose detection due to its sensing limitation. Interestingly, the usage of Cu/Chitosan exhibits good sensitivity and selectivity properties in which the angle shifting had been observed for all thicknesses of the thin films (Fig. 6(b)). At t=35nm, large-angle shifting about 1.30% and 1.25% were eventuated as the water changed to glucose solution (1.38 RIU and 1.53 RIU). At 36nm and 37nm of thicknesses, the sensor able to distinguish between these two concentrations of glucose in which the angle was shifted about 1.19%. Similarly, from 38nm until 42nm, the sensor also portrays its selective ability in which the SPR angle shifting about 0.65% from 44.97° to 47.09° was observed. The sensor unable to detect any changes as the Cu’s thicknesses were fixed between t=43nm to 47nm. At 48nm and 49nm, the sensor shows a good responsivity where the angles were slightly shifted, about 0.66%.

Figure 6: SPR angle shifting as sensing medium changed from water to glucose solution (1.38 RIU and 1.53 RIU) using different types of hybrid thin films (a) Ag/Chitosan (b) Cu/Chitosan

The sensitivity analysis between Ag/Chitosan and Cu/Chitosan in detecting glucose with different concentration is shown in Fig. 7. Evidently, the sensitivity of Cu/Chitosan SPR sensor is better than Ag/Chitosan. The latter one only able to distinguish different concentration of glucose when total hybrid
Figure 7: Sensitivity analyses of glucose with different concentration, 70mg/dl and 235mg/dl using hybrid thin films SPR sensor

film thicknesses were set between 50nm to 52nm with sensitivity, \( S = 2^\circ/\text{RIU} \). Note that, other than those thicknesses, the Ag/Chitosan unable to sense the presence of different types of glucose. The Cu/Chitosan SPR sensor offers better advantage than Ag/Chitosan in which its sensitivity performance was observed at all metal thin film thicknesses. The maximum sensitivity about \( 6^\circ/\text{RIU} \) was obtained when total thin film thicknesses were varied between 46nm to 49nm and 53nm and 56nm. The lowest sensitivity was resulted as the Cu/Chitosan thin films were set between 40nm and 42nm and from 47nm to 49nm with \( S = 4^\circ/\text{RIU} \).

In this study, there are few factors that need to be taken into consideration before concludes the best sensor’s sensitivity. The study should be started with the \( Q^2 \) value analysis to discover the stability of thin film as plasmonic materials. Excellent polynomial pattern of Cu/Chitosan with an average value of \( Q^2 = 0.9883 \) indicates that this hybrid thin films are more stable than Ag/Chitosan. The \( Q \)-factor analysis is significant to estimate the intensity of SPR signal because it constitutes the amount of light that has been absorbed by the material before converts it to SPP. Application of Ag/Chitosan appears to develop slightly better SPP excitation with 1.80% of SPR signal that stronger than Cu/Chitosan. Angle shifting indicates the responsivity of the sensor in detecting various sensing media. Obviously, the Cu/Chitosan able to discriminate three media, namely water, glucose with 1.38 RIU and 1.53 RIU at once validates the advantage of these hybrid materials in comparison with Ag/Chitosan. These results are further authenticated by the sensitivity analysis, where the Cu/Chitosan hybrid thin films appear to be the most sensitive and selective sensor with maximum \( S = 6^\circ/\text{RIU} \). In a whole, the development of SPR biosensor for the application of blood glucose detection can be developed with the deployment of Cu/Chitosan thin film with total thicknesses between 46nm to 56nm.

4. Conclusions
This study investigates the effect of metal thicknesses of hybrid Ag/Chitosan and Cu/Chitosan based SPR biosensor in detecting glucose with different concentration. The concentration’s values are similar with the concentration of glucose in human blood. The \( Q \)-factor analysis indicates the polynomial relationship between metal thin film thicknesses and the amount of SPP excitations. Cu/Chitosan SPR sensor with total thicknesses within the range from 46nm to 49nm and from 53nm to 56nm exhibits 67% better potential than Ag/Chitosan for biosensing applications due to its sensitivity and selectivity in differentiate glucose with dissimilar concentration. The maximum sensitivity of the Cu/Chitosan based SPR sensor is \( 6^\circ/\text{RIU} \). The Cu/Chitosan SPR sensor also offers a low material cost, easy to handle, cheap, miniaturized and can sense a very small concentration of glucose.
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