A nanolayered structure for sensitive detection of hemoglobin concentration using surface plasmon resonance

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Abstract

The objective of the proposed work is to design a biosensor that monitors hemoglobin (Hb) concentration using the combination of nanolayer, i.e., barium titanate (BaTiO₃) and antimonene based on surface plasmon resonance (SPR) technique. Antimonene is used here as bio-recognition element (BRE) layer to attach the Hb analyte through physical adsorption due to its hydrophilic nature, higher adsorption energy and larger active surface area. The use of BaTiO₃ adlayer (7 nm) just before antimonene is to enhance the refractive index (RI) sensitivity up to 1.90 times for the proposed SPR biosensor. The reason behind sensitivity enhancement is its high dielectric constant which enhances the electromagnetic field with in analyte medium. The performance of the biosensor is demonstrated with performance parameters namely sensitivity, detection accuracy (DA), figure of merit (FOM) and resolution. The proposed biosensor has potential to achieve much higher performance in terms of RI sensitivity of 303.83°/RIU, FOM of 50.39 RIU⁻¹ and resolution of 0.021 g/l in comparison with reported biosensors in the literature for detection of Hb concentration. Thus, based on the obtained results one can say that the proposed work unlocks a reliable sensing in the field of medical science to detect hemoglobin-related diseases in human being.

Keywords Hemoglobin (Hb) concentration · Resolution · Limit of detection (LOD) · Figure of merit (FOM) · Surface plasmon resonance (SPR) biosensor

1 Introduction

Hemoglobin (Hb) is a kind of protein of the red blood cells that is composed of four polypeptide subunits (protein chain), two alpha folded and two beta folded chains composed of different amino acids. Each chain possesses iron containing pigment (heme) essential for rapid binding of molecular oxygen [1]. It plays a fundamental role in

transporting molecular oxygen in the blood of vertebrates, from respiratory organs to the rest of the tissues and the organs [2]. The levels of Hb in blood in terms of shortage and saturation are the major biomarkers of diseases [3] such as anemia [4], leukemia, heart diseases [5] and hemoglobinuria [6]. Anemia is the state at which the concentration of Hb in blood is comparatively lower than normal to meet physiological needs. According to WHO, worldwide 42% of children below the age of 5 years and 40% of pregnant women are greatly affected by anemia [7]. The standard cutoffs for the Hb concentration of non-anemic children, men, non-pregnant women and pregnant women are 110 g/l, 130 g/l, 120 g/l and 110 g/l, respectively [8]. Various sensing mechanisms available for detection of Hb concentration, such as electrochemistry, optical, liquid chromatography, spectrophotometry and chemiluminescence, are capable to provide specific information from biological recognition and physicochemical transducers [9–13]. But, most of them follow time-consuming procedures and require number of devices installation, complex sample pretreatment process and fabrication process of electrodes (Fig. 1). Among all above-mentioned sensing mechanisms, optical sensing

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Fig. 1 Schematic diagram of proposed SPR biosensor

schemes especially surface plasmon resonance (SPR) was widely used to detect even smaller concentration of various biomolecules because of their non-invasive, label-free real-time detection and fast procedures with high accuracy and sensitivity [13]. In 1990, BIACORE commercially developed SPR-based devices for practical biosensing purpose [14]. Therefore, application of SPR biosensors has widened and greased the wheels of development in the medical field.

SPR biosensor can be suitably used for the competent detection of Hb concentration, which is an essential parameter for the diagnosis of blood-related diseases. The surface plasmon resonance condition may be achieved after proper matching of wave vector of p-polarized incident light coupled through prism, grating or waveguide with surface plasmon wave (SPW) [15, 16]. The resonance condition is very sensitive to change in RI of analyte medium after attachment of biomolecules onto biosensor surface [15–17]. The RI of the Hb acts as a biomarker of dysfunctions and pathologies caused by various diseases [18]. The light scattering and absorption properties of blood play an essential role for the detection of Hb concentration. The different concentrations of Hb exhibit different dispersion relationships owing to variation in chemical and biological composition of blood sample [16]. Consequently, the SPR condition gets satisfied at different resonance angle. Therefore, a reliable and simple SPR biosensor can be explored for regular monitoring of anemia like diseases. O Zhernovaya et al. have experimentally determined the RI of the human oxygenated and deoxygenated Hb for its different concentrations from 0 to 140 g/l at different wavelengths in visible range [19]. The group measured different concentrations of Hb using refractometer after mixing different concentrations of human Hb (lyophilized powder) purchased from Sigma-Aldrich in phosphate buffer saline (PBS) solution. As per experimental validation, the linear relationship between the concentrations

of oxygenated Hb and its RI was observed [19]. These experimentally measured RIs corresponding to different oxygenated Hb concentrations are used for this work [19]. Here, we proposed SPR biosensor to detect Hb concentration for monitoring its saturation and shortage levels.

However, conventional SPR biosensors based on single metal cannot be efficiently used for sensing of Hb concentration due to its smaller sensitivity and selectivity. The use of 2D nanomaterials as a BRE in conventional SPR biosensors can perform highly sensitive and selective attachment of analytes depending upon their molecular adsorption energies, electrical, optical and physical properties [15, 20]. 2D nanomaterials are not only used attachment of analytes, but these nanomaterials also increase the adsorption energy of photons to generate stronger SPR excitations than single metal film-based conventional SPR biosensor [15]. Hence, 2D nanomaterials have been used for wide medical applications such as sensing of DNA [21], disease biomarkers [22] and detection of biomolecules concentration with extremely high sensitivity owing to their larger specific surface area, abundant active sites for selective adsorption of analytes [12]. In 2013, Sharma et al. proposed a plasmonic biosensor that utilized gold film over the fused silica for the detection of Hb concentrations. The group obtained concentration sensitivity (S_c) ($0.005^\circ/\text{gl}^{-1}$) and resolution (0.18 g/l) for their work [16]. In 2016, Mohanty et al. have analyzed effect of nitrides in graphene-based SPR biosensor for the detection of hemoglobin, to enhance the concentration sensitivity (S_c) and resolution up to $0.286^\circ/\text{gdl}^{-1}$ or ($0.0286^\circ/\text{gl}^{-1}$) and 0.00350 g/dl or (0.035 g/l), respectively [17]. Recently, Kesavarz et al. used Au/graphene/ WS_2 SPR configuration for the monitoring the Hb level in human blood with maximum RI sensitivity (S_R) of $235.24^\circ/\text{RIU}$ [2]. The 2D nanomaterial graphene and WS_2 were used to enhance the sensitivity of SPR biosensor. A number of available 2D nanomaterials such as black phosphorus (BP), TMDCs and MXene are available, but some of them are chemically unstable and show weak interaction with the biomolecules [15, 20–23]. Among popular 2D nanomaterials, graphene shows shortcoming of zero bandgap, hydrophobicity and MoS_2 shows less conductivity which limits the biosensor performance [23]. BP shows 40 times higher molar response factor even greater than graphene and TMDCs, high charge carrier confinement and parts per billion sensing ability, but its susceptibility to oxidation limits its use for direct attachment of analyte in SPR biosensor [22, 23]. The 2D nanomaterial with large absorption energy and high work function is more favorable to be used as a BRE layer to enhance the biosensor performance. So, a new emerging 2D nanomaterial antimonene owing unique optical properties has been considered as the BRE layer of the proposed SPR biosensor. Its unique physical and optical properties such as sp^2 bonded honeycomb lattice structure, high carrier mobility,

remarkable stability and hydrophilicity make it better than other 2D materials [24]. It has been observed from the literature that the use of high RI adlayer such as silicon and metal oxides in SPR biosensor can further improve the sensitivity by enhancing the evanescent field with in sensing medium [15, 25]. Recently, tetragonal crystal-structured barium titanate (BaTiO_3), a perovskite material, has attracted attention of the researcher for sensing applications due to its remarkable optical properties such as its high dielectric constant value and smaller losses [26]. BaTiO_3 being a good photocatalyst absorbs the light to higher energy level, and this trapped light in the form of SPR excitation energy is transferred to metal layer due to the rapid charge injection in to the metal [26]. Thus, its uses in 2D nanomaterial configured SPR biosensors can provide high sensitivity and low FWHM which may not be obtained on using antimonene only. Hence, the use of antimonene for selective attachment of Hb analyte and adlayer of BaTiO_3 for enhancing the RI sensitivity can work well for the proposed biosensor for sensing of different concentrations of Hb. The nanolayers of BaTiO_3 and antimonene have been first time used in the present work for sensing of Hb concentration in blood plasma, which will be helpful to diagnose diseases associated with Hb concentration. Our foremost motivation behind this work is to present a SPR biosensor for sensing of Hb concentration with very high RI sensitivity as well as good resolution.

2 Design consideration

A five-layer Kretschmann's configured SPR biosensor has been proposed here. In this section, we have systematically discussed optical properties such as RIs and optimized thicknesses of different layers.

3 The RI of the constituent layers

The fluoride glass material-based BaF_2 prism is used as a coupling device which has advantages of increasing sensitivity over the other glass prisms. The RI of the BaF_2 prism considered for this work is 1.4733 [27].

The flat surface of BaF_2 prism can be coated with the 40-nm optimized silver (Ag) film for SPs generation using DC magnetron sputtering or high-power impulse magnetron sputtering (HiPIMS) technique [28]. The field distribution attenuation in Ag is high compared to gold (Au) film at the surface of metal. This indicates the Ag film provides much sharper dip in the reflectance curve than the Au film [29]. Therefore, Ag is preferable over Au metal to resolve the minor changes in RI of the analyte which improves the resolution of the proposed biosensor. But Ag suffers from oxidation problem which may be overcome by depositing high

RI BaTiO_3 over it by using sol–gel spin-coating approach [30]. Then, antimonene film obtained through liquid-phase sonication process from bulk antimony (Sb) can be electrophoretically deposited on the barium titanate as BRE layer [31]. Table 1 shows the RIs and optimized thicknesses of constituent layers used for proposed SPR biosensor.

The sensing layer of SPR configuration is PBS solution containing Hb analyte with different concentrations from 0–140 g/l. To prepare the sensing sample with different concentrations of oxygenated Hb, human Hb (in powdered form) concentrations from 20 to 140 g/l and sodium bicarbonate (15 g/l) need be dissolved in PBS solution [19]. Here, the PBS solution with zero concentration of Hb analyte is considered as reference solution or pure solvent referring to bulk RI. Experimentally measured RIs corresponding to different concentrations of oxygenated Hb in PBS solutions have been adapted from reference [19]. We have used experimental RI values of oxygenated Hb solutions indicating linear increment of RI with higher Hb concentrations for simulation purpose. However, when the actual experiment is to be carried out, an appropriate Hb sensitive buffer layer should be used [19, 32]. In the proposed work, there is no change in the SPR curve with the thickness of the sensing layer containing sensing samples, when it varies from 1 nm to $0.1\ \mu\text{m}$ due to large penetration depth into the sample layer [33]. Interaction of hemoglobin contained in PBS solution with the antimonene layer alters the phase-matching condition of SPR, which results in shifting of resonance angle with sensing layer RI (Δn_s) variation. This transformation may be witnessed through SPR curves plotted analytically using transfer matrix modeling, as shift in resonance angle or angular width of the SPR curve.

4 Modeling and performance parameters of the SPR biosensor

A transfer matrix method has been applied here to determine the reflectivity of the 5-layer proposed SPR model [34]. MATLAB simulation software has been used to perform analytical simulations for the biosensor modeling presented in our previous works [15, 21, 22]. Thus, SPR curves can be plotted for proposed angular interrogated Kretschmann configured

Table 1 Thicknesses and RIs of constituent layers used in proposed design

Constituent Layers	Thickness (nm)	RIs
Silver (Ag)	40	As per Drude model
Barium titanate (BaTiO_3) [42]	7	2.4042
Antimonene [43]	0.5	$1.40 + i \times 1.30$

biosensor by carrying out MATLAB simulations. The biosensor performance is assessed by evaluating primarily three performance parameters, i.e., sensitivity, resolution and figure of merit from SPR plots. The RI sensitivity (S_R) and concentration sensitivity (S_C) of the SPR biosensor can be calculated using Eqs. [2, 14, 15, 35],

$$S_R = \frac{\text{Change in Resonance angle}}{\text{Change in analyte RI}(Hb)} = \frac{\Delta\theta_{SPR}}{\Delta n_a} [\text{Deg./RIU}] \quad (1)$$

$$S_C = \frac{\text{Change in Resonance angle}}{\text{Change in analyte concentration}(Hb)} = \frac{\Delta\theta_{SPR}}{\Delta C_a} [^\circ/\text{gl}^{-1} \text{ or } ^\circ/\text{gdl}^{-1}] \quad (2)$$

where $\Delta\theta_{SPR}$ is the difference between resonance angle at zero concentration and 140 g/l. The resolution of SPR biosensor can be defined as the smallest detectable change in the concentration of the Hb analyte in the PBS solution [16, 17]. Its smallest value is desirable for sensing of smaller concentrations of analyte.

$$\text{Res.} \left(\text{in } \frac{\text{g}}{\text{l}} \right) = \frac{\text{Change in Hb analyte concentration}}{\text{Change in resonance angle}} = \frac{\Delta C_a}{\Delta\theta_{SPR}} \quad (3)$$

Here, the angular detection limit of SPR sensor is considered 0.001° for angular interrogation method [16].

The figure of merit (FOM) is the product of RI sensitivity (S_R) and detection accuracy (DA) and can be given as [15]

$$\text{FOM} = S_R \times \text{DA} \quad (4)$$

5 Results and discussion

The role and significance of each constituent layer of the proposed SPR design have been already described in subsection 2.1. Now, the performance analysis of proposed SPR design for different Hb concentrations along with optimization of constituent layers is presented in this section. It is very important to optimize the thicknesses of constituent layers in order to balance the photon absorption energy efficiency and energy loss of each layer [23]. The thicknesses of all constituent layer of the proposed SPR design are optimized in terms of minimum reflectance and change in resonance angle obtained for the proposed biosensor. The minimum value of reflectance tells the maximum coupling of incident light with the SPW, and change in resonance angle reflects the sensitivity of the proposed SPR biosensor. Now, Figs. 2 and 3 demonstrate the optimization of Ag, BaTiO₃ thicknesses and number of antimonene layers in terms of minimum reflectance and change in resonance angle. Figures 2 and 3 are plotted for conventional SPR, Ag/antimonene and proposed SPR at 1, 5 and 7 nm thicknesses of BaTiO₃ to compare the role of each constituent layer.

The plots shown in Fig. 2 also reflect the analytical investigations carried out for the effects of Ag and BaTiO₃ layer thickness on variation in resonance angle (θ_{res}), under the angular sensing performance at fixed wavelength $\lambda = 632.8$ nm. The thickness of Ag varies from 25 to 60 nm, and corresponding minimum reflectivity and change in resonance angle ($\Delta\theta_{\text{res}}$) are plotted in Fig. 2a and b, respectively. The optimization of thickness of Ag and BaTiO₃ layer corresponding to minimum reflectivity obtained is 40 nm and 7 nm, respectively, based on angular scan and also shown in Fig. 2a. The minimum reflectivity is soundly dependent

Fig. 2 a The minimum reflectivity and b change in resonance angle as function of thickness of silver (Ag) at 0, 1, 5 and 7 nm thicknesses of barium titanate (BaTiO₃)

Fig. 3 **a** The minimum reflectivity and **b** change in resonance angle as function of number of antimonene layers at 0, 1, 5 and 7 nm of BaTiO₃

on the thickness of Ag layer. Figure 2a shows that for fixed thickness of BaTiO₃ layer, in the beginning for smaller Ag thickness the minimum reflectivity decreases and reaches to the minimum value and then increases with further increment in Ag thickness. Figure 2b demonstrates maximum change in resonance angle for conventional, Ag/antimonene-based SPR and proposed SPR with 1 nm, 5 nm and 7 nm thickness of BaTiO₃ is 3.42, 3.44, 3.62, 5.00 and 6.38 degrees, respectively, corresponding to minimum reflectivity. Figure 2b shows that the highest change in the resonance angle is achieved with proposed biosensor (with 7 nm BaTiO₃) along with 40 nm thickness of Ag. Thus, the result indicates that both BaTiO₃ layer and antimonene layer act as the absorption layers. The BaTiO₃ layer absorbs the energy and transfers it to Ag layer to strongly enhance the SPs field in the sensing layer. Monolayer antimonene is desired for the proposed biosensor design to lessen the effective damping of SPs. Thus, further increment in antimonene layers can be overburdened by the inescapable energy loss [23, 24]. The effective SPR biosensor should show superior resonance angle shift as well as very low minimum reflectivity and less than 0.01 [36]. It is clearly observed from Fig. 2a and b that the optimized thickness of Ag should be in the range 33 to 40 nm and thickness of BaTiO₃ layer should be 7 nm. The thickness of Ag considered in this work is 40 nm.

Figure 3a and b shows the variation in minimum reflectivity and resonance angle shifts ($\Delta\theta_{\text{res}}$) with antimonene layers, respectively. From Fig. 3a, it is observed that the minimum reflectivity increases with number of antimonene layers and BaTiO₃ thickness. Figure 3b tells that the resonance angle shifts ($\Delta\theta_{\text{res}}$) are almost constant, without BaTiO₃ layer and for 1 nm thickness of BaTiO₃ layer. The resonance angle shift ($\Delta\theta_{\text{res}}$) increases a little bit till 3 layers of antimonene up to 5 nm of BaTiO₃, while it decreases further for more than 3 layers of antimonene. Thus, the

proposed SPR biosensor achieved highest resonance angle shift ($\Delta\theta_{\text{res}}$) at monolayer of antimonene at 7 nm BaTiO₃ thickness; further, it decreases with increase in number of antimonene layers due to inevitable energy loss [21]. At monolayer antimonene, the minimum reflectivity is lowest with highest change in resonance angle. Thus, antimonene is optimized at single layer for the proposed SPR biosensor.

The comparison of minimum reflectance, change in resonance angle and sensitivity for conventional SPR, proposed SPR biosensor with and without BaTiO₃ is presented in Table 2. The proposed SPR biosensor with 7 nm of BaTiO₃ for detection of Hb has much higher RI sensitivity (303.8313 Deg./RIU) as compared to conventional SPR (155.3807 Deg./RIU)- and Ag/Antimonene (159.173 Deg./RIU)-based SPR biosensor configuration.

Now, the proposed SPR biosensor is tested analytically for sensing of Hb concentration at optimized Ag, BaTiO₃ thicknesses and number of antimonene layers, in reference to experimental measured RI of oxygenated Hb at different concentrations [19]. Figure 4 depicts the SPR curves simulated at optimized layer thicknesses of proposed SPR design for sensing of different concentrations of Hb. The optimized thicknesses of Ag film, BaTiO₃ and antimonene considered for this plot are 40 nm, 7 nm

Table 2 Performance parameters of SPR biosensor configurations

SPR biosensor configurations	R_{min} (a.u.)	$\Delta\theta_{\text{SPR}}$ (Deg.)	RI sensitivity (Deg./RIU)
Conventional	0.222592	3.262995	155.3807
Ag/antimonene	0.04224	3.34264	159.173
with 1 nm BaTiO ₃	0.03715	3.52369	167.795
with 5 nm BaTiO ₃	0.00172	4.91655	234.122
with 7 nm BaTiO ₃	0.15337	6.38046	303.831

Fig. 4 SPR curves at different concentrations of hemoglobin (Hb)

and 0.5 nm, respectively, also marked in Table 1. It is clearly observed that the resonance angle (θ_{res}) significantly increases corresponding to large Hb concentrations in PBS solution, i.e., 80.15° for 0 g/l to 86.53° for 120 g/l. On increasing Hb concentration, the interaction between Hb biomolecules and antimonene increases due to larger surface-to-volume ratio and higher adsorption energies of antimonene, which results in incrementing the sensing layer RI. This disturbs the phase-matching condition of SPR and resulting in attainment of SPR condition at higher incident angle. The minimum reflectivity of SPR curves slightly increases with Hb concentration (0.016 a.u. for 0 g/l to 0.153 a.u. for 120 g/l). The inset of Fig. 4 clearly shows the linear increment of resonance angle (θ_{res}) for higher concentration of Hb.

The performance parameters of proposed SPR biosensor at 7 nm thickness of BaTiO₃ layer are shown in Fig. 5. The

variation in FWHM and minimum reflectivity is 6.22, 6.5, 6.65, 6.85, 7.03, 7.07, 6.83 and 6.03 degrees and 0.016, 0.008, 0.005, 0.0009, 0.0006, 0.010, 0.048 and 0.15 a.u., respectively, corresponding to linear variation in Hb concentration in the sample from 0 g/l to 140 g/l, shown in Fig. 5a. The minimum reflectance is observed to decrease towards zero at higher Hb concentrations and subsequently increase after 80 g/l, moving away from zero. The FWHM is another performance parameter which should be narrow to achieve the high figure of merit. In Fig. 5a, the FWHM increases with Hb concentration till 100 g/l, but further increments in Hb concentration narrow the FWHM. Now, Fig. 5b shows RI sensitivity and figure of merit corresponding to different concentrations of Hb at 632.8 nm wavelength. The RI sensitivity of biosensor is evaluated with reference to water sample with zero Hb concentration. Sensitivity is increased with an increment in Hb concentration. The figure of merit is the function of sensitivity and FWHM. It increases with high RI sensitivity and smaller FWHM. The RI sensitivity and figure of merit obtained are 251.95, 258.11, 268.46, 280.31, 293.35, 304.74 and 303.83 Deg./RIU and 38.76, 38.81, 39.19, 39.87, 41.49, 44.61 and 50.38 RIU⁻¹, respectively, corresponding to Hb concentration value of 20, 40, 60, 80, 100, 120 and 140 g/l. Thus, it may be concluded that the proposed SPR biosensor configuration achieves highest RI sensitivity of the order of $303.83^\circ/\text{RIU}$. The incremented values of minimum reflectivity and increasing trends for FWHM at higher concentrations of Hb (corresponding to higher RIs) are due to limitation of angular range [37]. Thus, the decrement in RI sensitivity, higher values of minimum reflectivity and FWHM for the proposed sensor after 80 g/l of Hb concentration are due to angular limitation of resonance angle when low RI prism is used [38]. SPR curves with larger minimum reflectance

Fig. 5 The performance parameter variation of proposed SPR biosensor **a** FWHM and minimum reflectivity vs. Hb concentration, **b** RI sensitivity and FOM vs. Hb concentration

(0.2 a.u.) should be discarded in determining the SPR angle, since a shallow resonance dip is not appropriate for precise and accurate detection practically [39]. So, in this work, we have considered the SPR curve with minimum reflectivity 0.15 at 1.355 RI of the Hb sample.

The resolution of biosensor calculated from Eq. (3) indicates that the smallest Hb concentration change can be sensed for the proposed biosensor. The resolution is one of the most essential parameters to determine the quality and precise measurement of concentration of Hb from the PBS solution. The resolution achieved for the proposed SPR biosensor is 0.021 g/l, which is an extensively very small value of Hb concentration. Therefore, the proposed SPR biosensor can measure the extremely small change in the concentration of Hb.

The great RI sensitivity and much smaller resolution are achieved by proposed SPR biosensor. Both essential parameters are dependent on the shift in resonance angle which is

Fig. 6 Simulated SPR curves for conventional, Ag/antimonene and proposed SPR biosensor with 1, 5 and 7 nm thickness of BaTiO₃ at 0 g/l and 140 g/l Hb concentration

much larger because of BaTiO₃ layer used for the proposed biosensor design. This is due to high RI of BaTiO₃ layer which enhances the field within sensing layer.

Figure 6 shows the variation in SPR curves from 0 to 140 g/l concentration of Hb for conventional and proposed SPR at different thickness of BaTiO₃ layer. The 0 g/l and 140 g/l Hb concentration is analogous to RI value of 1.334 and 1.355, respectively. The solid line indicates the SPR curves at RI-1.334 (i.e., 0 g/l), and dashed line indicates the SPR curves at RI-1.355 (i.e., 140 g/l). The SPR curves are plotted for $\Delta n_s = 0.021$, i.e., RI shift of sensing sample. On comparing SPR curves for conventional SPR (black line), Ag/antimonene SPR (red line) and proposed SPR at different BaTiO₃ layer thickness (blue, green and purple lines), it is clearly observed that minimum value of reflectivity reduces. Hence, after adding the antimonene layer, SPR curve achieved minimum reflectivity less than 0.05 a.u., due to high absorption energy and great molecule attachment (hydrophilicity) of antimonene that reduces the energy loss [24]. On adding BaTiO₃ layer, it is observed that the resonance angle (dip) is right shifted with increment in thickness of BaTiO₃ layer [26]. Hence, BaTiO₃ layer is responsible for the large shift in resonance angle owing to its large RI [26].

Table 3 shows the performance of proposed SPR biosensor for the different concentrations of oxygenated Hb in PBS solution. The minimum reflectivity, change in resonance angle and FWHM numerically evaluated from the SPR curves are shown here. The performance parameters are calculated using Eqs. (1), (2), (3) and (4). The highest RI sensitivity and FOM obtained are 303.83 Deg./RIU and 50.38 RIU⁻¹ for the proposed biosensor configuration.

Finally, COMSOL multi-physics simulation based on finite element method (FEM) is used to analyze SPs field pattern and normalized electric field distribution for the conventional, Ag/antimonene and proposed SPR biosensor [40]. Figure 7 shows the SPs field pattern and distribution at the interface of different layers. It is observed that SPs field distribution for conventional SPR biosensor at interface of Ag

Table 3 Performance parameters of proposed SPR biosensor at 7 nm thickness of BaTiO₃ for oxygenated Hb concentrations in PBS solutions

Oxygenated Hb Concentration (g/l)	$R_{\min}$ (a.u.)	FWHM (Deg.)	D.A. (Deg. ⁻¹)	$\Delta\theta_{\text{res}}$ (Deg.)	Sensitivity		FOM (RIU ⁻¹)
					S_R ($\Delta\theta_{\text{res}}/\Delta n$) (Deg./RIU)	S_C ($\Delta\theta_{\text{res}}/\Delta C_a$) (Deg./g l ⁻¹)	
20	0.0088	6.5	0.1538	1.0078	251.9582	0.0503	38.7628
40	0.0051	6.65	0.1503	1.5487	258.1175	0.0387	38.8146
60	0.0009	6.85	0.1459	2.4161	268.4626	0.0402	39.1916
80	0.0006	7.03	0.1422	3.3638	280.3196	0.0420	39.8747
100	0.0106	7.07	0.1414	4.4003	293.3544	0.0440	41.4928
120	0.0482	6.83	0.1464	5.4854	304.7499	0.0457	44.6193
140	0.1533	6.03	0.1658	6.3804	303.8313	0.04557	50.3866

and analyte shows higher amplitudes as compared to other two proposed SPR biosensors. Here, the use of BaTiO₃ and antimonene in proposed SPR biosensor shows the decrementing SPs field penetration in analyte region. But it shows the sufficient field strength used for sensing of smaller size biomolecules such as Hb (5 nm) [41].

Figure 8 shows the normalized electrical field distribution of evanescent field for conventional, Ag/antimonene and proposed SPR biosensor. At the antimonene/Hb sensing sample interface, the induced electric field is normalized by the incident electric field and z is the distance from interface. The peak field distribution appears at prism/Ag interface indicating the maximum excitation of the SPs, while it decays exponentially away from the sensing medium interface. Figure 8c clearly tells the sufficient field strength with in analyte region to sense Hb in PBS sample, if compared with field plot shown for conventional SPR biosensor shown in Fig. 8a.

The concentration sensitivity corresponding to change in concentration of Hb (0.045Deg./gl^{-1}) is much better as compared to SPR biosensors listed in Table 4. Table 4 shows the

comparison of the performance parameter of proposed work with existing biosensors for Hb concentration measurement. All above biosensors listed in Table 4 are evaluated by their sensitivity and precision (resolution) parameters. It is clearly observed from Table 4 that the proposed biosensor shows the smallest resolution and highest sensitivity (in terms of concentration and RI sensitivity) as compared to existing biosensors listed here. Hence, proposed biosensor is highly sensitive along with high resolution.

6 Conclusion

The measurement of Hb concentrations plays the essential role in the medical science-related research area to identify the diseases and new biomarkers at early stage of life-threatening diseases such as cancer, COVID-19 etc. Therefore, biosensor should be highly sensitive along with much higher resolution. To design enhanced performance of proposed SPR biosensor, first the thicknesses of Ag, BaTiO₃ and antimonene layers was optimized at 40 nm, 7 nm and 1

Fig. 7 The SPs field distributions of the **a** conventional, **b** Ag/antimonene SPR and **c** proposed SPR biosensor

Fig. 8 The normalized electric field distribution **a** conventional, **b** Ag/antimonene SPR and **c** Ag/BaTiO₃/antimonene-based SPR biosensor

Table 4 Comparison of the sensitivity and resolution of proposed SPR biosensor with the existing biosensors

References	Year	Technique	λ nm	Sensitivity	Resolution
A.K. Sharma [16]	2013	SPR	706.5	0.005 °/g ⁻¹	0.18 g/l
G. Mohanty et al. [17]	2016	SPR	632.8	0.286 °/gdl ⁻¹	0.00350 g/dl
V.A. Popescu et al. [44]	2019	SPR	1530	–	0.0036 g/dl
A.K. Goyal et al. [35]	2020	BSW	380	0.0133 °/g ⁻¹ and 69 °/RIU	–
A. Keshavarz [2]	2020	SPR	632.8	235.24 °/RIU	–
Proposed work		SPR	632.8	303.83°/RIU and 0.045 °/g ⁻¹	0.021 g/l

layer, respectively. This novel work helped to achieve high concentration sensitivity of 0.045°/g⁻¹ and good resolution of 0.021 g/l, and enabling the proposed biosensor is capable to sense even very small change of Hb concentration at 632.8 nm characteristic wavelength. It also provides the high RI sensitivity of 303.83 Deg./RIU, which is much better than existing prominent SPR biosensors for sensing of Hb concentrations in PBS solutions. The proposed SPR biosensor allows the wide operating range for sensing of Hb concentration within the practical limit. Thus, the proposed biosensor with good resolution and high sensitivity can be used to identify hemoglobin concentration in blood sample too.

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