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Abstract. A label-free technique for the detection of triacylglycerides by a localized surface plasmon resonance (LSPR)–based biosensor is demonstrated. An LSPR-based fiber-optic sensor probe is fabricated by immobilizing lipase enzyme on silver nanoparticles (Ag-NPs) coated on an unclad segment of a plastic clad optical fiber. The size and shape of nanoparticles were characterized by high-resolution transmission electron microscopy and UV–visible spectroscopy. The peak absorbance wavelength changes with concentration of triacylglycerides surrounding the sensor probe, and sensitivity is estimated from shift in the peak absorbance wavelength as a function of concentration. The fabricated sensor was characterized for the concentration of triacylglyceride solution in the range 0 to 7 mM. The sensor shows the best sensitivity at a temperature of 37°C and pH 7.4 of the triacylglycerides emulsion with a response time of 40 s. A sensitivity of 28.5 nm/mM of triacylglyceride solution is obtained with a limit of detection of 0.016 mM in the entire range of triacylglycerides. This compact biosensor shows good selectivity, stability, and reproducibility in the entire physiological range of triacylglycerides and is well-suited to real-time online monitoring and remote sensing. © 2017 Society of Photo-Optical Instrumentation Engineers (SPIE)
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1 Introduction

The estimation of metabolites such as triacylglycerides in whole blood is important in clinical diagnostics. Triacylglycerides are triesters of glycerol and fatty acids with typical chain lengths of 12 to 18 carbon atoms and degrees of unsaturation varying between 0 and 6. These are a primary component of vegetable and animal lipids and, hence, a critical constituent of the human diet. An imbalance of blood metabolites is considered to be one of the most severe threats to human health and is related to the high risk of several serious illnesses. Triacylglyceride concentration in serum of a healthy person is in the range of 150 to 190 mg/dl. When triacylglyceride level is higher than 200 mg/dl, it causes hypertriglyceridemia; 200 to 499 mg/dl is considered a high level and severe high level is greater than 500 mg/dl.[1] Hypertriglyceridemia can be atherogenic and is a prevalent risk for cardiovascular diseases, specially, in the current situations of obesity and insulin resistant syndromes. In the past decade, various types of biosensors have been developed for estimation of blood metabolites. In general, a biosensor consists of two components, one is a biomolecule, which is highly specific (recognition element) toward an analyte, and second is a transducer, which converts the molecular recognition event into a quantifiable signal. The lipase enzyme is one such biomolecule that can be immobilized on a solid substrate by different methods and used to detect the presence of the analyte triacylglyceride (TG) in a solution.

Various methods are available for triacylglycerides detection like porous silica-based potentiometric pH-dependant triacylglyceride biosensor,[2] potentiometric and micromechanical triacylglyceride biosensor,[3] pH-based sensor,[4] amperometric sensors based on multienzymes,[5–8] SPR-based sensor,[9] etc. For these reported triacylglyceride biosensors, leaching of enzyme is an associated problem and also often a sophisticated fabrication process makes the sensor fabrication tedious.

Hence, there is a still need to develop a device, which is handy and robust, requires minimal preprocessing of sample, provides quick response, and offers label-free detection. Localized surface plasmon resonance (LSPR)[10,11–18]–based immunoassay satisfies all these criteria. Localized surface plasmons (LSPs) are charge density oscillations confined to metal nanoparticles. Excitation of LSPs by light results in scattering and absorption, which depends on the type of metal, the size and shape of the nanostructure, refractive index of the surrounding environment, and wavelength of light. The change in transmitted intensity by extinction due to absorption and scattering as a function of wavelength is measured by monitoring the absorbance spectra. A shift in the peak absorbance wavelength is a measure of the surrounding environment. The combination of LSP and fiber-optic technology has led to the development of a platform having high sensitivity, label-free detection, simplicity, and ease of applicability for sensing purposes.

In this study, we report the fiber-optic based triacylglycerides sensor utilizing LSPR realized by metal nanoparticles for the first time. The probe was prepared by attaching silver

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nanoparticles (Ag-NPs) over a small length of an unclad optical fiber core and immobilizing lipase enzyme (biorecognition layer) over the surface of the metal nanoparticles. The sensor operates in the wavelength modulation scheme, in which the peak absorbance wavelength is measured with respect to the changing concentration of triacylglyceride solution. The presence of triacylglyceride molecules in the environment of the sensing region changes the refractive index and thickness of the biorecognition film due to the hydrolization of triacylglycerides by lipase enzyme.[19] As a consequence, the absorbance spectra of the metal nanoparticles change significantly. The characterization of the present sensing probe has been carried out using different concentrations of triacylglyceride solution ranging from 0 to 7 mM (which corresponds to 0 to 619.78 mg/dl). The performance of the fiber-optic probe was optimized by varying the dipping time in Ag-NPs solution for attaching the nanoparticles to the glass surface of the fiber and varying the concentration of the enzyme solution during immobilization. The response time, repeatability, and specificity of the sensor were verified and the effect of temperature on the enzyme response was also studied. The fast response and requirement of a very small volume of sensing analyte make the proposed sensor suitable for commercialization. A theoretical calculation of the extinction cross section of a spherical Ag-NP in water has also been carried out to compare with the measured results for the synthesized Ag-NPs dispersed in water and estimate their radii.

2 Material and Methods

2.1 Materials

Silver nitrate ($AgNO_3$), polyvinyl pyrrolidone (PVP), and ammonia used for preparing Ag-NPs were purchased from Fisher Scientific, LOBA Chemical and CDH Biochemical, New Delhi, respectively. 3-Aminopropyl triethoxysilane (3-APTES) for surface modification of Ag-NPs was purchased from Sigma Aldrich. The reagents sodium di-hydrogen orthophosphate and di-sodium hydrogen orthophosphate for preparing phosphate buffer solution (PBS) were purchased from Fisher Scientific Pvt. Ltd., India. Triolein was purchased from Sigma Aldrich for the preparation of the triacylglycerides samples. Plastic clad silica optical fiber of 600-μm core diameter with 0.37 numerical aperture (NA) was purchased from Fiber Guide Industries. Distilled water used for the preparation of sample was purchased from Millipore. The preparation of lipase (Lip 11) enzyme from Yarrowia lipolytica MSR 80 was carried out by the method reported in the literature.[20] Silver (99.9% pure) was purchased from a local vender and no further purification of chemicals was performed.

2.2 Synthesis of Silver Nanoparticles

The Ag-NPs were synthesized by the chemical reduction method. PVP, which works as the reducing and particle stabilizing agent, and $AgNO_3$ were used as starting materials.[21,22] Two aqueous solutions with 43 g of PVP dissolved in 30 ml of distilled water and 0.03 g of $AgNO_3$ dissolved in 30 ml of distilled water were prepared. The two solutions were mixed to get a white solution, in which PVP: $AgNO_3$ solution was in the ratio of 6:1. The mixture was kept in a beaker on a magnetic stirrer with 1300 rpm at room temperature. Aqueous ammonia was then added drop by drop into the mixed solution

of PVP and $AgNO_3$ until the milky solution becomes transparent. This step results in the formation of small particles of Ag^+ ions. The solution was then left in an oven for about 40 h at a temperature of 160°C. At this temperature, the small particles grow into larger particles. A yellowish transparent solution is formed, which indicates the formation of Ag-NPs. The solution of Ag-NPs was stored in the refrigerator at 4°C before it was further used. The confirmation of the formation of Ag-NPs was carried out by characterization techniques such as high-resolution transmission electron microscopy (HRTEM) and UV–visible spectroscopy as discussed in Sec. 3.1.

### 2.3 Preparation of Triacylglyceride Emulsions

For the preparation of triacylglycerides emulsions, a concentrated stock solution of triacylglyceride (50 mM) was prepared by emulsifying 8.85 g of triolein with 2 g of gum acacia in 200 ml of sodium PBS of pH 7.4 in a homogenizer for 15 min. Different concentrations of solutions with triacylglyceride solutions ranging from 0 to 7 mM were prepared by diluting the stock solution of triacylglyceride (50 mM) with sodium PBS. 1 mM of triacylglyceride solution is equivalent to 88.54 mg/dl, and hence, the 0- to 7-mM concentration range is equivalent to 0 to 619.78 mg/dl, which covers the physiological range of triacylglycerides in human blood. The refractive index of the triacylglycerides solution of different concentrations showed no variation within the measurement accuracy of the Abbe refractometer with a resolution of 0.001.

2.4 Experimental Setup

The schematic diagram of the measurement set-up is shown in Fig. 1. The optical setup consists of a polychromatic light emitting source (tungsten halogen lamp—Avalight-Hal) with a spectral emission range of 300 to 1100 nm, the AvaSpec-3648 fiber-optic spectrometer, a microscope objective, and a flow cell into which the sensing fiber probe is fixed. Light from the tungsten halogen lamp is focused at the input end of the optical fiber probe by the microscope objective to couple light into the fiber. The other end of the optical fiber is coupled to the spectrometer for measurement of the absorbance spectra. The flow cell is mounted on three-dimensional-translational stage to maximize coupling of the light into the fiber. The sensing region in the flow cell is exposed to triacylglycerides solutions of different concentrations ranging from 0 to 7 mM. Peak absorbance wavelengths corresponding to the varying concentrations are extracted from the measured absorbance spectra.

2.5 Fabrication of Sensing Probe

The probe was fabricated on a 15-cm plastic clad silica fiber of 600-μm core diameter and 0.37 NA. A plastic clad fiber was used specifically to facilitate removal of the fiber cladding from a 1-cm length in the middle section of the fiber length. Prior to the immobilization of Ag-NPs onto the surface of the unclad section of the fiber, the fiber was cleaned by distilled water and acetone. The cleaned fiber was coated with Ag-NPs by dip coating method, in which the fiber was dipped vertically into a long narrow cylindrical vessel containing the Ag-NPs solution. The Ag-NPs-coated fiber was dipped in 10% ethanolic solution of 3-APTES[19,23] for 2 min to create the amino functional group ($-NH_2$) on the surface of Ag-NPs. The functionalized fiber was then immersed into lipase enzyme solution
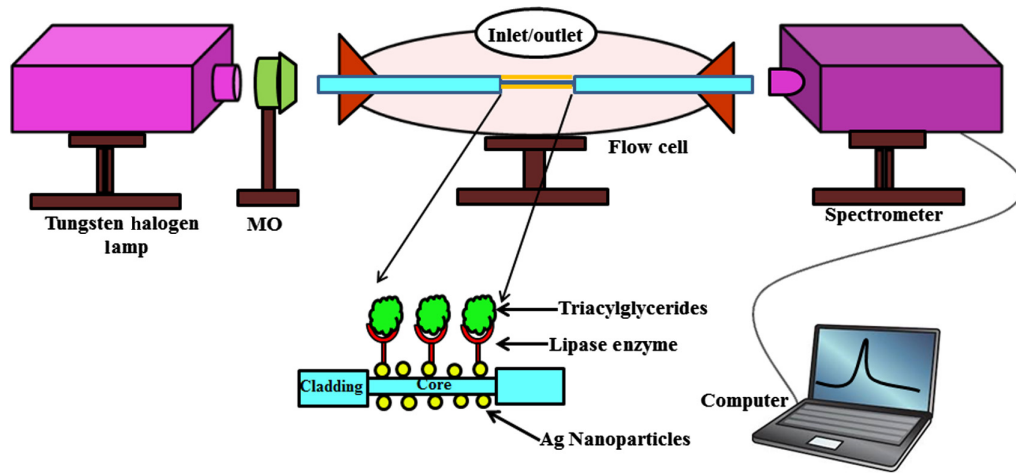
Fig. 1 Schematic diagram of the experimental setup for characterizing the LSPR-based fiber-optic biosensor.

made by dissolving typically 40 mg of lipase (Lip11) enzyme in 10 ml of PBS. The lipase enzyme is immobilized by the interaction with amine groups ($-NH_2$) of APTES on the surface of Ag-NPs-coated fiber. The schematic representation of immobilization of enzyme on the optical fiber probe is shown in Fig. 2. The fiber with this biorecognition (enzyme) layer was washed with PBS to remove the excess adsorbed enzyme on the surface and then the final probe was kept in a refrigerator (at ~4°C) for 24 h. It is observed that this step results in better binding of the enzyme. However, for measurements, the probe was used at temperatures varying from 25°C to 45°C.

3 Results and Discussion

3.1 Characterization of Ag-NPs

#### 3.1.1 Transmission electron microscopy analysis

Morphology and structural imaging of the nanoparticles were also observed with Tecnai G2 S-Twin HRTEM using an accelerating voltage of 100 kV. HRTEM is a microscopy technique, in which a high-energy electron beam is incident on the samples. This technique provides fine structural information about the sample even as small as an atom and about its crystalline or amorphous nature. For observing the HRTEM, a sample of the Ag-NPs was deposited on a copper grid and allowed to dry at room temperature. Bright field imaging mode was used to analyze the Ag-NPs sample. The thicker region of the sample with the higher atomic number appears dark, whereas the bright region appears when there are no silver particles in the path of the high-energy electron beam as shown in Fig. 3(a). This TEM image analysis shows that the sample is composed of almost spherical nanoparticles with varying diameters from ~30 to 50 nm.

Figure 3(b) shows the more resolved image of the Ag-NPs. Fringes can also be clearly observed in Fig. 3(b), atoms are at the dark lines and lattices are at the bright lines, which indicate the crystalline structure of nanoparticles.
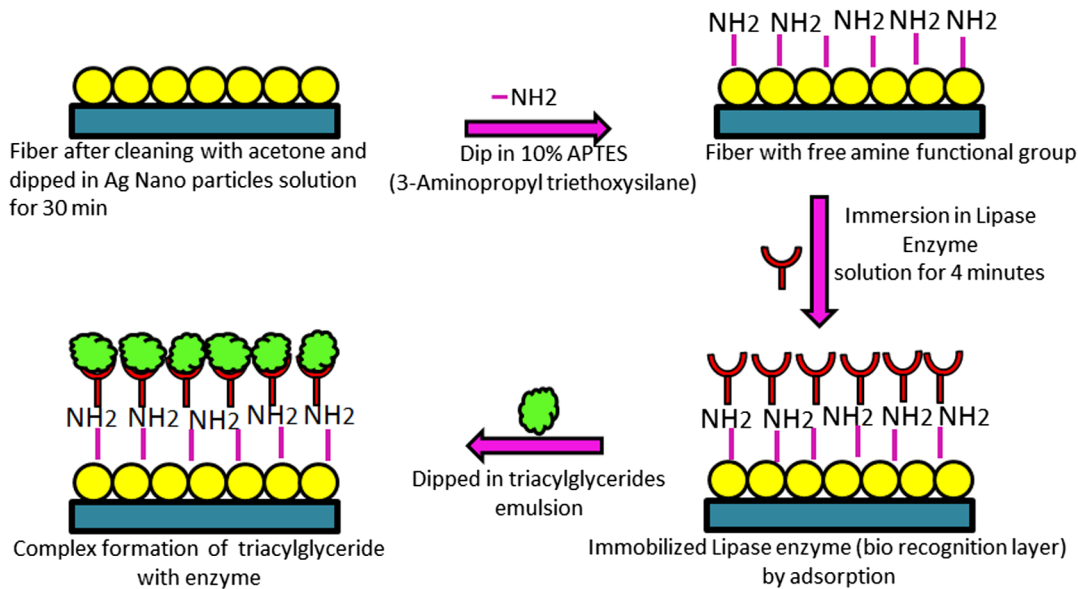
Fig. 2 Schematic representation immobilization of lipase enzyme to create the biorecognition layer on the optical fiber probe.
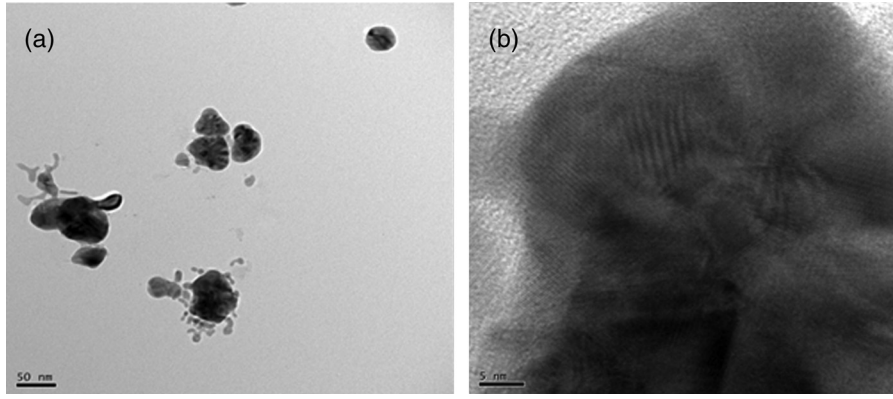
Fig. 3 (a) TEM image showing morphology of Ag-NPs sample at the scale of 50 nm. (b) TEM image of the sample at the scale of 5 nm.

3.1.2 UV Spectroscopy analysis

UV–visible spectroscopy is one of the most widely used techniques for structural characterization of nanoparticles. The absorbance spectra strongly depend on the shape, size, material, and environmental medium of the metal nanoparticles. The UV–visible transmission spectrum of the Ag-NPs solution was recorded by a spectrometer. Small spherical nanoparticles exhibit a single surface plasmon resonance peak, whereas anisotropic particles rod and triangle like structures show more peaks in UV–visible range due to the transverse and longitudinal excitation of plasmons in a nanorod.[24] The orange line shown in Fig. 4(b) represents the result of the experimentally measured

extinction cross section for the synthesized Ag-NPs with a peak at 438 nm and full width half maximum (FWHM) of 125 nm. In the recorded absorption spectra, only one peak was seen as shown in Fig. 4(b), which is a characteristic of Ag-NPs of spherical shape.[21]
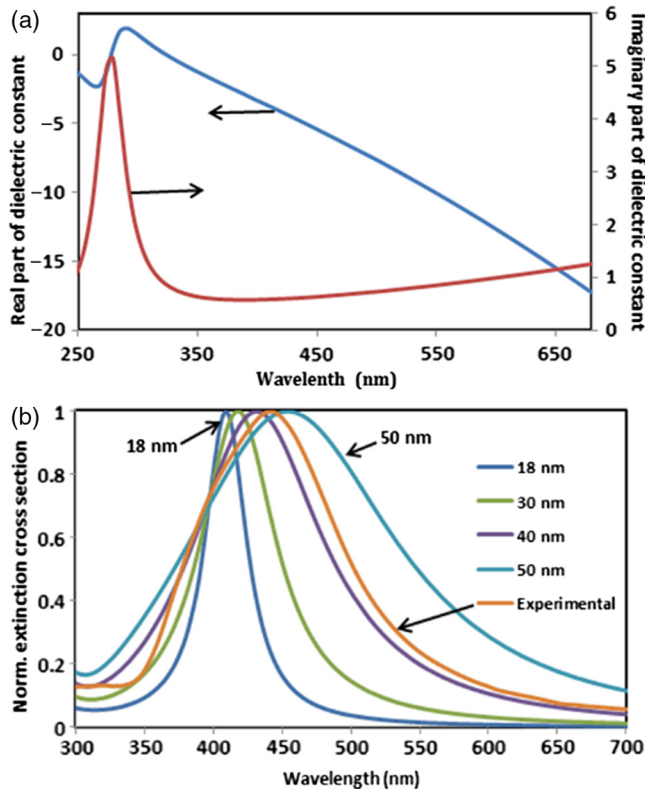
3.1.3 Theoretical estimation

In order to compare with the experimental response of nanoparticles, we also calculated the extinction cross section of a single nanoparticle. The extinction cross section (σ_{ext}) represents the total loss of energy in transmission, which is equal to the sum of scattering cross section (σ_{scat}) and absorption cross section (σ_{abs}). Hence, the extinction cross section can be written as

$$\sigma_{ext} = \sigma_{abs} + \sigma_{scat}. \tag{1}$$

For a spherical Ag-NP, the extinction cross section can be calculated by use of the following expression:[25]

$$\sigma_{ext} = \frac{2\pi}{\lambda}\mathrm{Im}(\alpha) \quad \alpha = 3V\varepsilon \frac{1 - \theta^2 \frac{(\varepsilon_m + \varepsilon)}{40}}{\frac{\varepsilon_m + 2\varepsilon}{\varepsilon_m - \varepsilon} - \theta^2 \frac{(0.1\varepsilon_m + \varepsilon)}{4} - \theta^3 \frac{2}{3} i\varepsilon^{3/2}}, \tag{2}$$

where V is the volume of the nanoparticle, ε and ε_m are the dielectric constant of surrounding medium and silver, α is the polarizability of NP, and $\theta = 2\pi R/\lambda$ is the size parameter. The variation of the dielectric constant with wavelength for silver metal, $\varepsilon_m = \varepsilon_{mr} - j\varepsilon_{mi}$, used in the calculations is plotted in Fig. 4(a) and corresponds to that given by Rakic et al.[26]

Figure 4(b) shows the spectrum of the normalized extinction cross section of a single Ag-NP of radii varying from 18 to 50 nm with water as the surrounding medium. From theoretical calculation, the extinction peaks are obtained between wavelength 431 and 455 nm for NP of radii changing from 40 to 50 nm with FWHM varying from 107 to 170 nm. Since the experimental result shows a peak at 438 nm with FWHM of 125 nm, it can be concluded that the radii of the synthesized Ag-NPs lie in range of 40 to 50 nm and the spread in the experimental measured extinction cross section is due to the dispersity in size of Ag-NPs.

Fig. 4 (a) Real (blue) and imaginary (red) part of dielectric constant of Ag. (b) Normalized absorbance spectra of Ag-NPs solution measured experimentally and theoretical normalized extinction cross section of single NP of radius 18 to 50 nm.

3.2 Characterization of the Fabricated Probe

Immobilization of lipase enzyme on the Ag-NPs coated on the optical fiber was confirmed by assay using *p*-nitrophenyl palmitate (PNP).[27] The lipase enzyme was immobilized on the Ag-NPs-coated fiber and the same fiber was dipped one by one in each of the four different cuvettes containing equal volumes of PNP solution for 10 min at a temperature of 37°C and reaction in each cuvette was stopped by using triton. The optical density (OD) of the reaction was observed by spectrophotometric analysis at a wavelength 410 nm. The OD obtained in each cycle, considering the first cycle, i.e., (0.21) as 100% are shown in Fig. 5. Negligible leaching of the enzyme was observed, showing that the property of native lipase on the Ag-NPs-coated fiber remains unaltered after multiple uses.

For the characterization of the probe, different concentrations of triacylglyceride solution ranging from 0 to 7 mM were poured into the flow cell one by one and the absorbance spectrum was recorded by the spectrometer. All the spectra were recorded 40 s after pouring of the solution into the flow cell. The absorbance spectra obtained for different concentrations of triacylglyceride solutions are shown in Fig. 6(a). This figure clearly shows that the peak absorbance shifts toward higher wavelength with an increase in the concentration of triacylglycerides in the solution.

Figure 6(b) shows the restructured absorbance spectrum for a clear representation of the shift in absorbance peak wavelength. The region of low absorbance is magnified in the inset. It may be mentioned that the spectra in this region appears noisy because the source emission power is relatively low in this spectral range. The shift in peak absorbance is due to the fact that immobilized lipase enzyme environment on the Ag-NPs-coated fiber changes on interaction with the triacylglycerides molecules of the solution. First, the lipase enzyme forms a complex with triacylglycerides and then in the presence of water the enzyme hydrolyses each ester bond of the triacylglycerides and produces diacylglycerides followed by monoacylglycerides, glycerol, and fatty acids. With an increase in the concentration of triacylglyceride in the solution, the refractive index and thickness of biorecognition layer surrounding the Ag-NPs increases. Consequently, absorbance spectrum and the peak absorbance wavelength change and a shift of 77 nm in peak absorbance wavelength are observed with the variation in the concentration
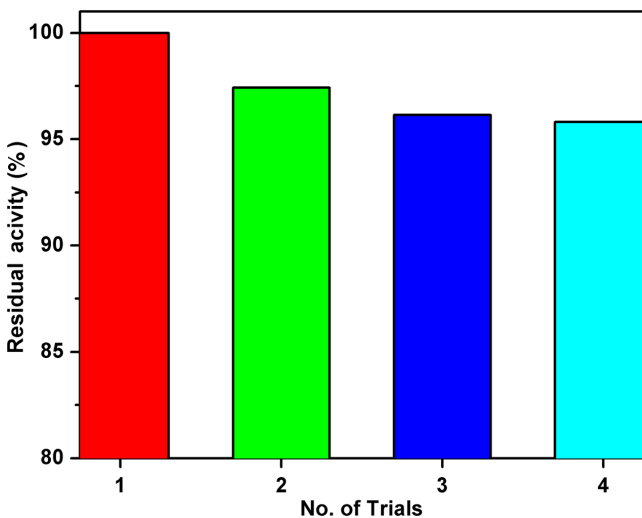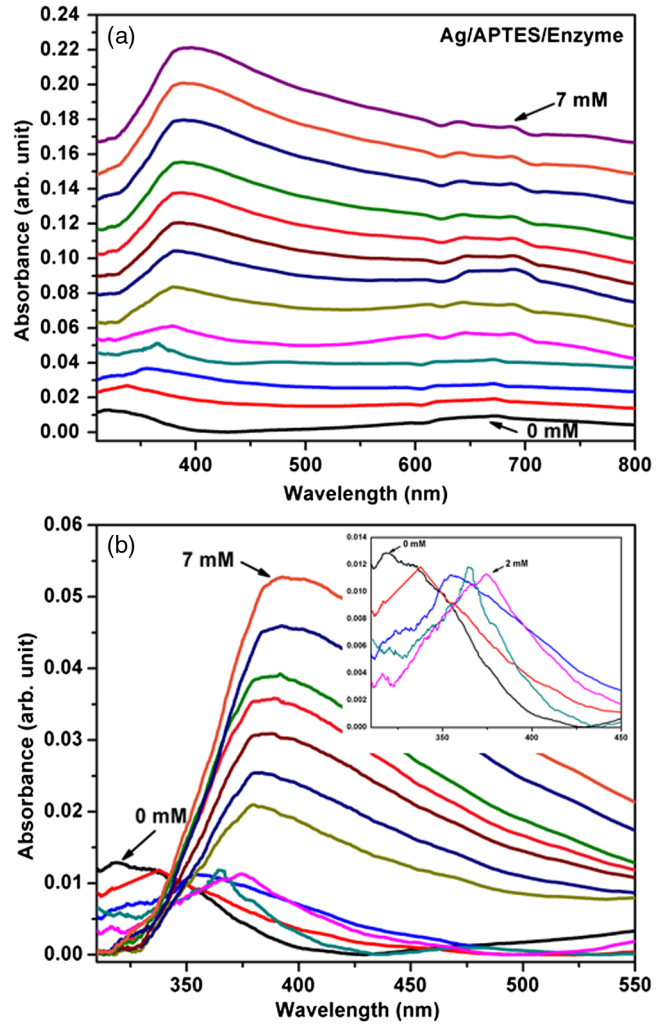

Fig. 6 (a) Absorbance spectra of the sensor for 0 to 7 mM concentration range of the triacylglycerides solution at pH 7.4 and temperature 37°C. (b) Rescaled absorbance spectrum of the senor.

of triacylglyceride solution from 0 to 7 mM. The shift in peak absorbance wavelength with triacylglycerides concentration plotted in Fig. 7(a) is not linear and saturates to a maximum value $\Delta\lambda_{max}$ for a given probe. The curve can be well fitted to the equation

$$\Delta\lambda = \Delta\lambda_{max}(1 - \exp^{-0.617C}), \qquad (3)$$

where $\Delta\lambda_{max}$ and C are the maximum shift in peak absorbance wavelength and concentration of triacylglycerides in mM in the solution, respectively. The rate of change in peak absorbance wavelength with concentration of triacylglycerides is higher at lower concentrations and gradually saturates to a value $\Delta\lambda_{max}$. This is due to the availability of sufficient active sites of the enzyme at lower concentrations of triacylglyceride. For high concentrations of triacylglycerides, all the active sites are used and no further change is possible.

3.2.1 Sensitivity

Sensitivity is an important performance parameter of any sensor. Sensitivity S is defined as the shift in peak absorbance wavelength per unit change in the concentration of triacylglyceride

Fig. 5 Variation in OD with number of trials.
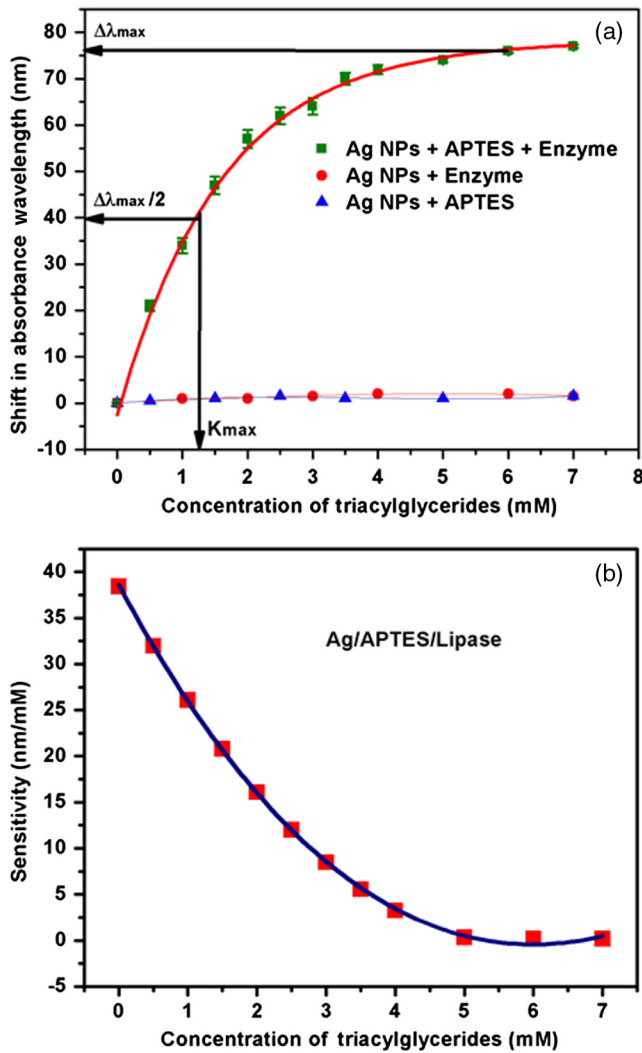
Fig. 7 (a) Variation of shift in peak absorbance wavelength versus concentration of triacylglycerides in pH 7.4 solution at 37°C standard deviation. Also plotted are the shifts in peak absorbance wavelength with only the functionalized nanoparticles and immobilization with functionalized without APTES. (b) Variation in sensitivity of the biosensor with the concentration of triacylglycerides.

solution around the sensor probe and can be calculated from the slope of the curve shown in Fig. 7(a) as

$$S = 0.617 \Delta \lambda_{\max} C \exp^{-0.617C}. \tag{4}$$

Figure 7(b) shows the variation of sensitivity as a function of the concentration of triacylglycerides solution. The present sensor probe is highly sensitive (28.5 nm/mM) at low concentrations of triacylglycerides solution but as the concentration of triacylglycerides approaches saturation, the sensitivity of the sensor deceases. The sensor can be used for the range of 0 mg/dl to approximately 440 mg/dl, which is the physiological range of triacylglycerides in human blood. Error bars have also been shown in Fig. 7(a), which takes into account the resolution of the spectrometer (0.33 nm), measuring pipette, weighting machine, and the size of the error bars can be reduced by using a spectrometer of better resolution.

3.2.2 Optimization of the sensor probe

The sensitivity of the optical fiber sensor probe is related to the immobilizing conditions such as the concentration of lipase enzyme solution and dipping time of the fiber into the enzyme solution. The amount of immobilized enzyme on the fiber surface is determined by the concentration of enzyme solution and immobilization time. It was observed that the sensor performance changes as the dipping time of the sensor probe into the enzyme solution (4 mg/ml in PBS) varies from 1 to 6 min. The best response of the sensor was observed for a dipping time of 4 min in the enzyme solution. The sensor probe was also optimized in terms of concentration of lipase enzyme solution by varying the concentration from 2 to 6 mg/ml at a dipping time of 4 min. It was observed that the sensor shows maximum response (shift in peak absorbance wavelength 77 nm) for 4 mg/ml concentration of enzyme solution with 4 min dipping time. For lower concentrations of enzyme solution, the sufficient enzyme is not immobilized on the fiber surface to hydrolyze the triacylglycerides. While for an increase in the concentration of lipase enzyme solution or with an increase in dipping time, the amount of the immobilized enzyme onto the nanoparticles gets saturated and deformation of the active sites of enzyme occurs.

Response of the sensor was also studied for the varying incubation time from 1 to 3 min in the APTES solution, which creates the amine group on the Ag-NPs-coated fiber to bind the lipase enzyme. The response of the sensor was found to be maximum for an incubation time of 2 min in APTES solution with a 4 mg/ml enzyme solution.

The response of the sensor also depends on the nanoparticles attached to the fiber surface in dip coating. The dipping period of the Ag-NPs solution was varied from 10 min to 1 h and the absorbance spectra were measured for probes fabricated with each sample. The best response with changing triacylglycerides concentration was observed when a dipping time 30 min was used to attach the nanoparticles. This is due to the fact that there is no significant attachment of Ag-NPs to the fiber surface for a dipping time less than 30 min, whereas an aggregation of nanoparticles is attached on the fiber surface for dipping times greater than 30 min. Aggregation of NPs produces broadening of the absorbance spectra and reduces the sensitivity of the sensor probe.[15] This can be seen from the absorption spectra recorded at different dipping times as shown in Fig. 8.

3.2.3 Control experiments

As a control experiment, it was also attempted to immobilize lipase enzyme on the Ag-NPs-coated fiber without functionalizing with APTES. The probe was immersed in triacylglyceride solution ranging from 0 to 7 mM and absorbance spectrum was measured by spectrometer. It was observed that no significant shift in peak absorbance wavelength occurs as also shown in Fig. 7(a). This implies that lipase enzyme did not directly bind to the Ag-NPs surface, and hence it is necessary to functionalize the Ag-NPs surface prior to lipase immobilization.

We also studied the response of the probe without immobilization of lipase enzyme on the Ag-NPs layer. No change in absorbance wavelength was observed when the probe was immersed in different concentrations of triacylglyceride solutions ranging from 0 to 7 mM, as also shown in Fig. 7(a). Therefore, it can be concluded that the change in absorbance spectrum and peak wavelength was caused by the occurrence
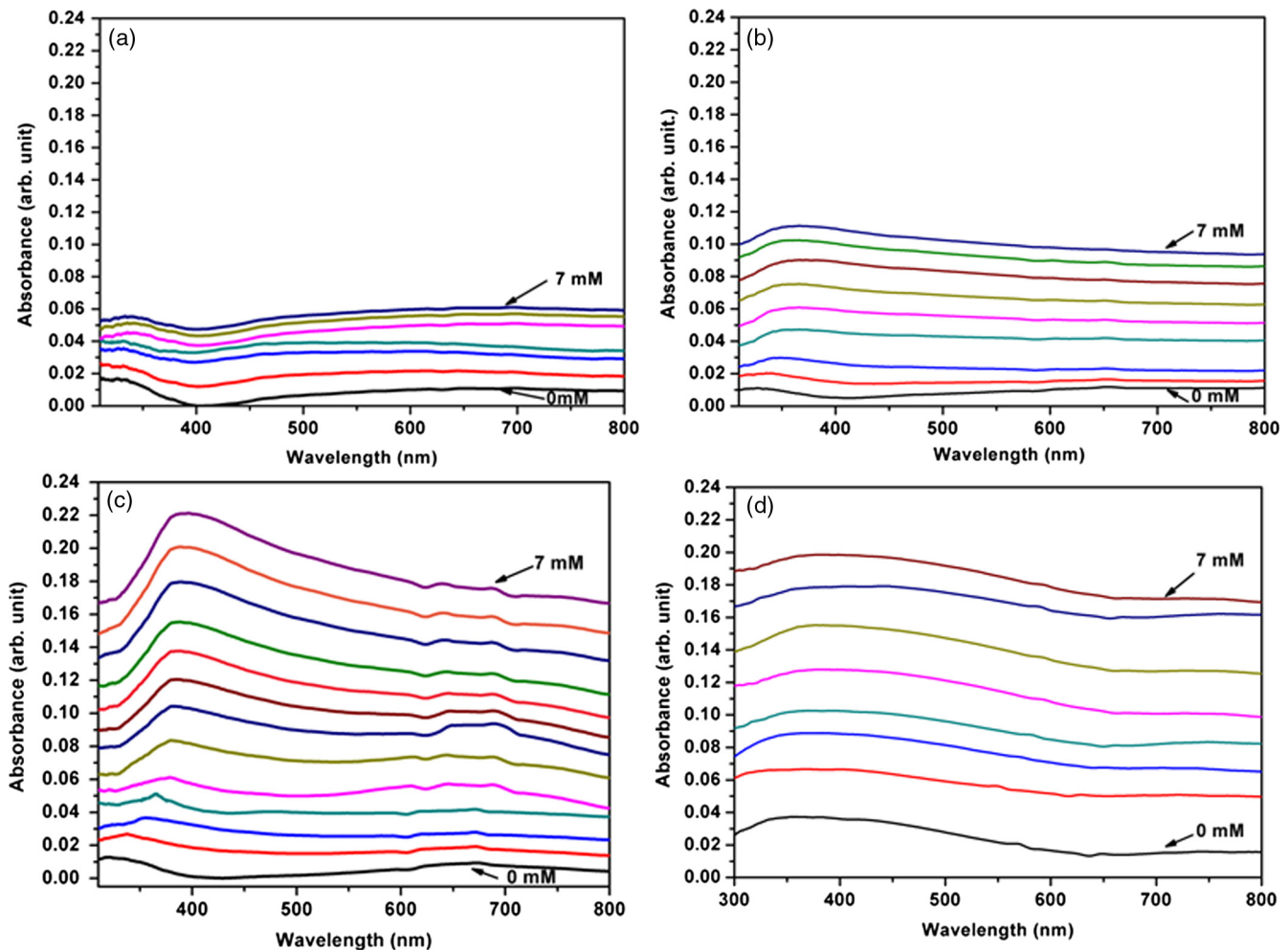
Fig. 8 Absorption spectra recorded at different dipping time (a) 10 min, (b) 20 min, (c) 30 min, and (d) 50 min in Ag-NPs solution.

of binding between the analyte and biorecognition layer on the Ag-NPs surface and not by a change in the refractive indices of the triacylglyceride solutions.

3.2.4 Effect of temperature

We also studied the effect of temperature on the performance of the sensor because the reactivity of enzyme with triacylglyceride emulsions is strongly affected by the temperature. Experiments were carried out for temperature of solutions varying from 25°C to 45°C. It was found that sensitivity of the probe is maximum at a temperature of 37°C. This implies that lipase enzyme shows maximum activity at the normal human body temperature of 37°C, which is the good agreement with earlier published results.[20]

3.2.5 Interference study

Another important issue that was checked to confirm the selectivity of the sensor, i.e., whether the lipase functionalized probe is affected by other substances like urea, glucose, ethanol, and ascorbic acid, which are usually present in human blood. To study this, interferents like urea, glucose, ethanol, and ascorbic acid at their physiological value were added into the triacylglyceride solutions of 2 and 4 mM concentration, and the shift in peak absorbance spectrum was recorded as shown in Table 1. It

can be seen that response of the sensor is not affected by the interferents present in the triacylglycerides solution. Thus, the proposed sensor is highly specific toward triacylglyceride.

3.2.6 Stability, repeatability, and response time of the sensor

The response of the sensor with time was also studied by observing the change in peak absorbance wavelength with time from 5 s to 1 min. It takes about 30 to 40 s to reach the true peak absorbance wavelength as shown in Fig. 9, or the response time of the present sensor is confirmed to be ∼40 s.

The stability of the sensor was checked by allowing the solution to remain in the vicinity of the sensor probe for 1 min and recording the peak absorbance wavelength after every 10 s following the 40 s of response time. The values of peak absorbance wavelength were observed to be almost stable for the period, confirming the stable response of the sensor.

To demonstrate the repeatability of the sensing probe, experiments were performed choosing the triacylglyceride concentration of 5 mM. The experiment was started by immersing the sensor probe in 0 mM concentration and recording the peak absorbance wavelength of the spectrum. Then, the probe was dipped in 5 mM solution and the corresponding peak absorbance wavelength was recorded after 40 s, which is the response

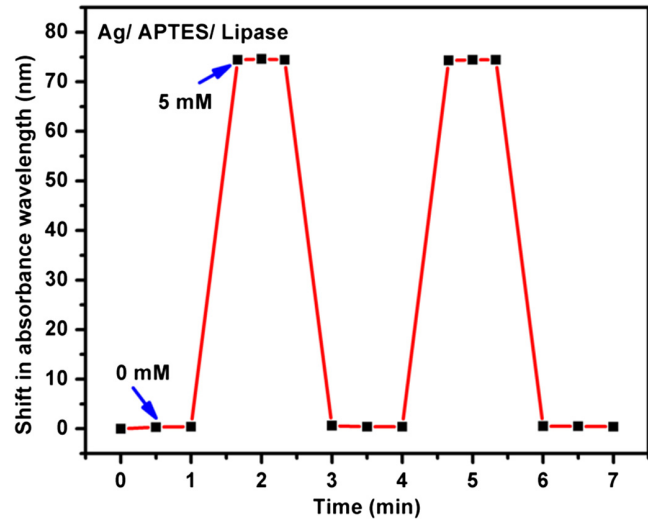**Table 1** Shift in peak absorbance wavelength of the sensor in presence of interferents.

Analyte (TGs) concentration (mM)	Additions/interferents	Shift in peak absorbance wavelength (nM)
2	NIL	57
2	Urea (42 mg/ml)	57.34
	Glucose (150 mg/ml)	
	Ascorbic acid (1 mg/ml)	
	Ethanol (80 mg/ml)	
4	NIL	72
4	Urea (42 mg/ml)	72.67
	Glucose (150 mg/ml)	
	Ascorbic acid (1 mg/ml)	
	Ethanol (80 mg/ml)	
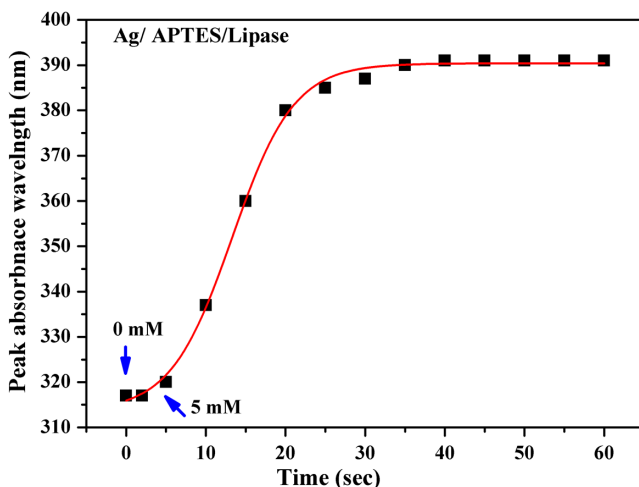
Fig. 9 Shift in peak absorbance wavelength with the time during hydrolysis of the triacylglycerides by lipase.

time of the sensor. The 5 mM solution was removed from the flow cell and again, the peak absorbance wavelength was recorded for the 0 mM concentration. No change from the earlier recorded value was observed, showing the repeatability of the sensor. A number of such cycles were carried out for checking the repeatability of the sensor. Figure 10 shows the very good repeatability of the sensor. Hence, the present lipase immobilized probe not only has good sensitivity but also holds good stability, repeatability, and low response time.

3.2.7 Reproducibility

The reproducibility of the present sensor was studied. The sensor probe was replicated for the same concentration of APTES and lipase and the same immobilization steps. The peak absorbance wavelength and shift in absorbance wavelength with the

Fig. 10 Variation in shift of peak absorbance wavelength of the biosensor with time during presence (5 mM) and absence (0 mM) of triacylglycerides solution around the probe to study the repeatability of the probe.

concentration of triacylglyceride are within the standard deviation value of 10%. This implies that the sensor probe is highly reproducible.

3.2.8 K_m value

Michaelis constant (K_m) is defined as the analyte concentration, in which the reaction rate is half its maximum value. In this case, the reaction rate is proportional to the peak absorbance wavelength. We have estimated the K_m value of the present sensor to be 1.3 mM from the graph in Fig. 7(a) between peak absorbance wavelength and corresponding triacylglyceride concentrations. This value is lower than that of the sensors reported in the literature. The lower K_m value implies that the immobilized lipase exhibits high catalytic activity, and hence, the fabricated sensor shows a high affinity toward triacylglyceride.

3.2.9 Limit of detection

Limit of detection (LOD) of a sensor is defined as the minimum concentration of analyte, which can be detected by the sensor with good accuracy. It can be defined by the ratio of the resolution of spectrometer to the sensitivity of the sensor as

$$LOD = \frac{\Delta\lambda_s}{S},$$

where S is the sensitivity of the sensor, and $\Delta\lambda_s$ is the resolution of spectrometer. It may be noted that the resolution of spectrometer is 0.33 nm, the LOD of the present sensor is 0.016 mM (1.4 mg/dl) near the lower concentration range of triacylglyceride solution. This LOD for triacylglyceride detection of the proposed sensor is lower than that for earlier reported sensors.

A comparison of the sensitivity, K_m value, response time, and LOD of various sensors reported in the literature for the detection of triacylglyceride using different techniques is tabulated in Table 2.

Table 2 Comparison with performance parameters of various triacylglycerides sensors reported in the literature.

Method	Sensitivity	K_m (mM)	Response time	LOD (mM)
Electrochemical CV[28]	00.57 μA mM^{-1} cm^{-2}	2.51	3 s	0.2
Lipase/NPG/GCE-based CV[29]	0.30 μA mg^{-1} dl cm^{-2}	0.12	Not reported	0.03
Long period fiber grating[19]	0.5 nm/mM	4.20	1 min	0.2
Potentiometric[4]	1 mM	Not reported	20 min	0.01
Amperometric[5,7,30]	0.05 μA/mg/dl[30]	Not reported	4 s	0.11
	19.29 μA mM^{-1} cm^{-2} (Ref. 7)	8.76	40 s	0.2
	Not reported[5]	5	30 s	0.11
SPR-based sensor[9]	3.17 nm/mM	3.5	1 min	0.1
LSPR [present work]	28.5 nm/mM	1.3	40 s	0.016

4 Conclusion

LSPR-based immunoassay on an optical fiber provides a platform for rapid and cost effective detection of the triacylglyceride. Detection of the triacylglyceride using nanoparticles-coated fiber-optic sensor is being reported for the first time. The advantage of using LSPR sensor is that it can be easily fabricated through a chemical process rather than using expensive equipment such as vacuum coating machine, as required for the thin film-coated SPR sensor preparation. LSPs are excited by the light travelling in the fiber and are responsible as the transduction mechanism for the presented triacylglyceride biosensor. The shift in absorbance spectra due to the increase in the refractive index and thickness of the biorecognition layer in the presence of triacylglycerides is used as the sensing parameter. The sensor is capable of detecting concentration of triacylglyceride with a sensitivity and resolution of 28.5 nm/mM and 1.4 mg/dl, respectively, in the range of triacylglyceride concentration in human blood. The designed sensor using Ag-NPs-coated optical fiber offers various advantages cost effectiveness, high sensitivity, good resolution, and response time. It is also suitable for real-time applications with online monitoring and remote sensing.

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