Biosensors and Bioelectronics 79 (2016) 693–700

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Long period fiber grating based sensor for the detection of triacylglycerides

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ARTICLE INFO

Article history:
Received 24 September 2015
Received in revised form
10 December 2015
Accepted 24 December 2015
Available online 30 December 2015

Keywords:
Long period fiber grating
Biosensing
Triacylglycerides
Covalent binding
Lipase

ABSTRACT

In this paper, stable, label free enzyme based sensor using long period fiber grating (LPG) is described for the detection of triacylglycerides. A stable covalent binding technique for lipase enzyme immobilization on an optical fiber is reported. An active and stable attachment of the functional group of the enzyme on the fiber surface is achieved using this method. Enzyme immobilization is confirmed by Scanning Electron Microscopy (SEM) and Raman Spectroscopy. The stability is confirmed by lipase p-nitrophenyl palmitate (PNP) assay. In contrast to widely used amperometric based biosensor, where a number of enzymes are required, only one enzyme, namely, lipase is required in our sensor. The sensor shows optimum response within one minute at a temperature of 37 °C and pH of 7.4. The sensor is based on the shift in resonance wavelength of the LPG transmission spectrum due to the interaction of triacylglycerides with the enzyme. The biosensor is highly specific towards triacylglycerides and is unaffected by the presence of many other interfering substances in serum. Interaction between the bio-molecules and the long period grating surface is also modeled theoretically using a four layer model for the LPG fiber with the bio-recognition layer and the results obtained are consistent with experimentally obtained results. The sensor shows a high sensitivity of 0.5 nm/mM and a low detection limit of 17.71 mg/dl for the physiological range of triacylglycerides in human blood.

1. Introduction

Triacylglycerides (TGs) are derived by esterification of three hydroxyl (–OH) groups of glycerol with three molecules of fatty acids, which play an important role in metabolism as energy source and transporters of dietary fat. During an energy crises adipocyte cells release the triacylglycerides to provide energy. An increased level of triacylglycerides indicates the risk of heart disease like coronary heart disease and hyperlipoprotenimia. Triacylglycerides level in the blood of a healthy person varies in the range of 150–190 mg/dl. Levels around 200 mg/dl are considered border line while those from 200 to 499 mg/dl are high and above 500 mg/dl correspond to a severe situation (http://en.wikipedia.org/wiki/Triglycerides). Therefore, a sensitive and selective determination of triacylglycerides level in blood and food is becoming increasingly important for clinical diagnosis. Various sensors are available for triacylglycerides detection, e.g. amperometric sensors (Minakshi and Pundir, 2008; Narang et al., 2010a, 2010b; Rejeb et al., 2007; Winartasaputra et al., 1982), potentiometric sensors (Reddy et al., 2001; Setzu et al., 2007), micromechanical sensors (Fernandez et al., 2009) and pH based sensor (Vijayalakshmi et al., 2008).

In the amperometric sensors, the enzyme is co-immobilized on a collagen membrane, cellulose acetate (CA), polyvinyle chloride (PVC), egg shell membrane and Prussian blue (PB) modified screen printed electrodes. The collagen membrane based sensor (Winartasaputra et al., 1982) has a drawback of a long preparation time of almost 3 days, long response time and difficulty in storage stability. CA (Minakshi and Pundir, 2008) and PVC (Narang et al., 2010a) membrane based sensors have a major disadvantage of desorption or leaching of enzymes during washing. In addition, the PVC based amperometric sensor requires four enzymes, i.e., lipase, glycerol kinase (GK), glycerol-3-phosphate oxidase (GPO) and horseradish peroxidase (HRP) to generate current. Egg shell membrane (Narang et al., 2010b) based sensor has a low detection limit of 25 mg/dl. Prussian blue (PB) modified screen printed electrodes based sensor (Rejeb et al., 2007) also requires three

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http://dx.doi.org/10.1016/j.bios.2015.12.089
0956-5663/

enzymes, i.e., lipase, glycerol dehydrogenase and NADH oxidase. In porous silicon based potentiometric biosensors (Reddy et al., 2001; Setzu et al., 2007), immobilization is carried out by physical adsorption method which also has the drawback of leaching of the enzyme. The micromechanical sensor (Fernandez et al., 2009) has a requirement of micromachining for fabrication while the pH based sensor (Vijayalakshmi et al., 2008) using magnetic nano particles requires a customized field effect transistor (FET) device. Hence, there is a need of new materials and methods for improving enzyme based triacylglycerides detection.

More recently, there has been a lot of interest in the use of optical fibers in sensor technology. Popular sensing fiber optic configurations include, Surface Plasmon based sensors (Rajan et al., 2006; Bhatia and Gupta, 2011; Baliyan et al., 2013), fiber Bragg grating (Sridevi et al., 2015) and long period grating based sensors (Baldini et al., 2012; Bosch et al., 2007; Chiavaioli et al., 2014; Deep et al., 2012; Erdogan, 1997; Falciai et al., 2001; Partridge et al., 2014; Tripathi et al., 2012; Yang et al., 2015). In this paper, we report a long period fiber grating (LPG) based biosensor for sensitive and label free detection of triacylglycerides.

A long period fiber grating (LPG) is a single mode fiber with a periodic modulation of the refractive index induced in the core by ultra violet (UV) exposure as shown in Fig. 1a. The period, Λ, is of the order of hundreds of micrometer and the refractive index variation can be typically written as

$$n(z) = (n_{co}+\Delta n) + \Delta n \sin\left(\frac{2\pi}{\Lambda} z\right) \quad (1)$$

The periodic modulation couples light from the core mode to different co-propagating cladding modes of the optical fiber at discrete wavelengths determined by the following phase matching equation

$$\lambda_m = \Lambda\left(n_{co} - n_{cl}^{(m)}\right) \quad (2)$$

where, $n$co and $n_{cl}^{(m)}$ are effective indices of the core mode and the mth cladding mode respectively. When broad band white light propagates through an LPG, a number of resonance dips occur in the transmission spectrum (Erdogan, 1997) at these wavelengths. The cladding mode is a mode guided by cladding–ambient interface and hence, the effective index $n_{cl}^{(m)}$ changes with change in ambient refractive index and this results in a change in the transmission spectrum. Similarly, changes in temperature, pressure and strain change the transmission spectrum and hence, LPGs are being used to sense refractive index, temperature, pressure and strain. More recently, the sensing capability of long period fiber gratings coated with nanoscale bio-recognition molecules has been extended to its use as a biosensor. If a bio-recognition layer is interfaced on the cladding surface, changes in the thickness and refractive index of this layer will also change the transmission spectrum. Various LPG chemical sensors and biosensors

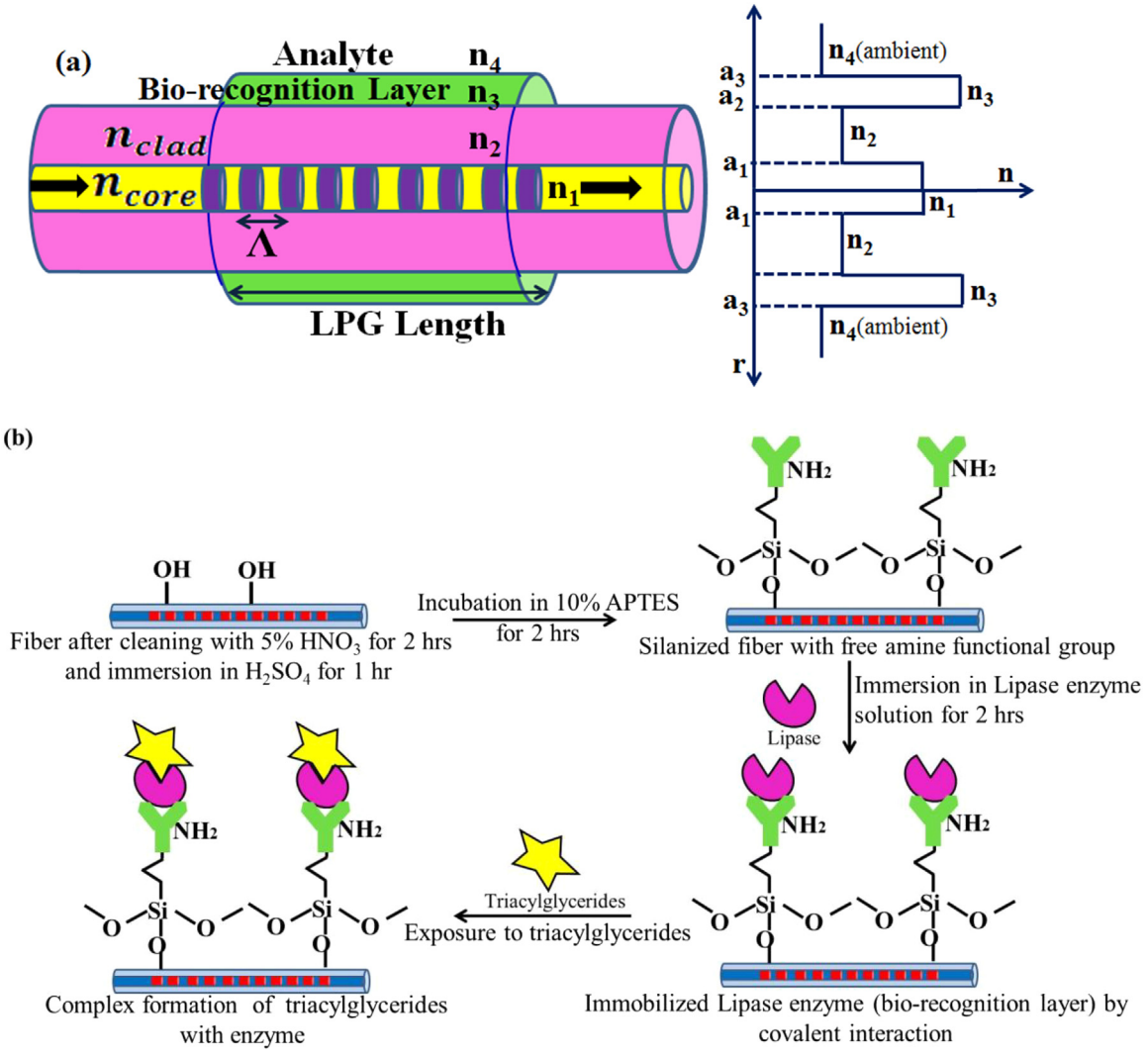


Fig. 1. (a) Schematic representation of the LPG fiber sensor probe (b) Immobilization of enzyme to create the bio-recognition layer on the optical fiber probe.

based on this phenomenon have been reported in literature (Baldini et al., 2012; Bosch et al., 2007; Chiavaioli et al., 2014; Deep et al., 2012; Erdogan, 1997; Falciai et al., 2001; Partridge et al., 2014; Tripathi et al., 2012; Yang et al., 2015). For immobilization of enzyme (bio-recognition molecules) on the optical fiber, various methods like adsorption, ionic bonding, electrostatic self-assembly technique, cross-linking and covalent bonding (Hanefeld et al., 2009) are available in literature. Out of these, covalent binding technique, which allows stable and active sites of enzyme on the optical fiber, is most effective. In this paper, we have prepared the sensing probe by covalent immobilization of lipase enzyme (Lip 11) for the detection of triacylglycerides on an optical fiber in which a long period grating has been fabricated. The immobilization of the enzyme on a glass fiber was confirmed by lipase p-nitrophenyl palmitate (PNP) assay, Scanning Electron Microscopy (SEM) and Raman spectroscopy. Change in refractive index and thickness of the interfacial bio-recognition layer occurs due to the enzyme- substrate complex formation and hydrolysis of triacylglycerides into glycerol and fatty acids. Due to this, a change in resonance wavelength occurs in transmission spectrum of the LPG and the shift in resonance wavelength can be correlated with the analyte concentration. The response time, reproducibility and specificity of the sensor were checked and the effect of temperature and pH of phosphate buffer on the enzyme response was studied. The sensor phenomenon was also theoretically studied using a four layer model for the LPG, i.e., core (with index modulation), cladding, few nanometer thick high index bio-recognition layer and ambient analyte solution.

2. Materials and methods

2.1. Materials

Nitric acid and sulfuric acid were used for cleaning and activating the hydroxyl group on the fiber surface respectively. The reagents sodium dihydrogen orthophosphate and di-sodium hydrogen orthophosphate for phosphate buffer solution (PBS) preparation were purchased from Fisher Scientific Pvt. Ltd., India. 3-Aminopropyl triethoxysilane (APTES), which is used for silanization of the fiber, and triolein, which is used for the preparation of triacylglyceride samples, were purchased from Sigma Aldrich.

2.2. Preparation of triacylglyceride emulsion

First, 100 ml of 0.2 M phosphate buffer of pH 7.4 was prepared by mixing 39 ml of 0.2 M sodium dihydrogen orthophosphate and 61 ml of 0.2 M di-sodium hydrogen orthophosphate (Nigam and Ajjagari, 2007) then diluted it by addition of 300 ml of de-ionized water to prepare 50 mM solution of pH 7.4. A concentrated triolein emulsion (Baliyan et al., 2013) of 50 mM was prepared by emulsifying 8.85 g of triolein with 2 g of gum acacia in 200 ml of sodium phosphate buffer of pH 7.4. The triolein emulsion of 50 mM can be diluted to prepare different concentrations ranging from 0.5 mM to 7 mM. 1 mM of triacylglycerides solution is equivalent to 88.54 mg/dl and hence the 0.5 mM to 7 mM concentration range corresponds to the physiological range of triacylglycerides level desired to be detected in human blood. Refractive indices of all the solutions of different concentrations were measured by an Abbe refractometer and were found to be identical, i.e., 1.333, upto a measurement accuracy to the third decimal place.

2.3. Fabrication of the LPG

The LPG was fabricated on a photosensitive Boron-Germanium co-doped optical fiber by the popular point to point method by use of a krypton fluoride (KrF) pulsed excimer laser (Taraxion FLEX-500-I writing system) at an operating wavelength of 248 nm. Optimal laser parameters are a pulse repetition rate of 200 Hz, pulse duration of 15 ns and peak pulse energy of 3 mJ. The fiber and laser were fixed on a computer operated high resolution translation stage. The fixed fiber was exposed to the UV radiation, point to point, by moving the laser by the period of 581 μm. For period $\Lambda = 581$ μm, resonant coupling occurs to the $m = 4$ cladding mode in the given fiber at λ_0=1545 nm. This result is a dip in the transmission spectrum at λ_0=1545 nm. The transmission spectrum of LPG was observed continuously during the process by connecting one end of the fiber to a broad band white light source and other end to an optical spectrum analyzer. At each exposure magnitude of the periodic refractive index change Δn is enhanced. The strength of coupling from the core mode to the resonant co-propagating cladding mode depends upon the magnitude Δn of the refractive modulation. Therefore, the process was repeated until an adequate Δn is attained to give a significant dip in the transmission spectrum at the resonant wavelength λ_0=1545 nm.

2.4. Immobilization of the lipase enzyme (bio-recognition layer) on the LPG surface

Covalent immobilization of enzyme on glass fiber surface has been shown to give satisfactory results (Deep et al., 2012; Hanefeld et al., 2009). We also used covalent immobilization of lipase enzyme for the preparation of the bio-recognition layer on the fiber surface using following steps:

(i) The fiber was first cleaned by immersing it in a 5% nitric acid at a temperature of 90 °C for a duration of 2 h and then rinsed with ethanol and deionized water.
(ii) In the next step, the cleaned fiber was dipped in sulfuric acid for 1 h at room temperature (25 °C) for activating the hydroxyl group on the fiber surface and subsequently dried. This process increases the hydroxyl groups availability, i.e., creates a hydrophilic environment for silane attachment.
(iii) The fiber was then incubated in 10% aminopropyl triethoxysilane (APTES) $H_2N(CH_2)_3Si(OC_2H_5)_3$ solution in ethanol for a duration of 2 h. This results in a silanized fiber with free amine functional groups ($-NH_2$) on its surface.
(iv) The incubated fiber was then rinsed with water and ethanol to remove non-covalently adsorbed amine groups.
(v) Next, the Lipase (Lip11) enzyme was immobilized by covalent interaction between amine groups on the fiber and –COOH group of enzyme by immersing the silanized fiber into the enzyme solution (4 mg/ml) in phosphate buffer solution of pH 7.4 for 2 h.
(vi) Finally, the LPG with this bio-recognition layer was washed with PBS to remove unbound (excess) enzyme and then let it dried in air by evaporation.

The schematic representation of immobilization of enzyme on the optical fiber probe is shown in Fig. 1b. The LPG transmission spectrum was monitored at different steps of the functionalization of the LPG and immobilization of lipase at room temperature as shown in Fig. 2. The resonance wavelength shows a shift as the cladding surface is modified. The transmission spectrum after immobilization at 37 °C is also shown in the Fig. 2. The resonance wavelength is a strong function of temperature, hence it is necessary to maintain a constant temperature for sensing purpose.

2.5. The experimental setup

The LPG with the bio-recognition lipase layer was clamped inside a special design temperature stabilized U-groove which forms the

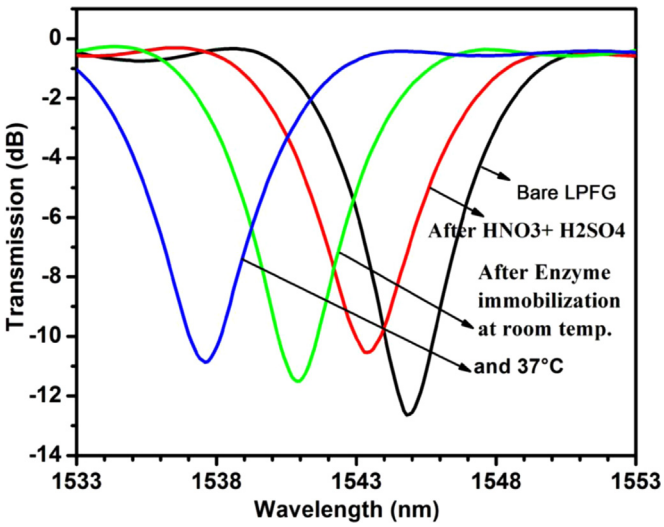


Fig. 2. Transmission spectrum of the LPG at each stage of immobilization of enzyme on the sensing region.

flow cell. The open cell U-groove is made of dimensions 25 cm × 0.5 cm × 0.5 cm in the center of the piece of aluminum block of dimensions of 25 cm × 3 cm × 3 cm. A thermocouple is inserted in aluminum block as close as possible to the flow channel and connected to a thermometric feedback control unit which is used to control the temperature of the entire system. One end of the optical fiber is connected to a white light source (AQ-4305) and other end is connected to an optical spectrum analyzer (AQ-6370). The fiber was clamped on both sides in U-groove to keep it straight throughout the experiment. Photograph of the measurement setup is shown in Fig. 3. The LPG sensor probe was then exposed to different concentrations of triacylglyceride emulsion ranging from 0 mM to 7 mM, i.e., 0–619.78 mg/dl. Optical spectrum was recorded by the OSA after 1 minute of settling time. After recording the spectrum, analyte solution was dipped out and probe was rinsed copiously with phosphate buffer until the initial spectrum was obtained.

3. Experimental results and discussion

3.1. Experimental

As stated above, the lipase enzyme was successfully immobilized on the long period fiber grating by the covalent binding technique. To confirm the possibility of covalent immobilization of lipase enzyme on the glass optical fiber, first the enzyme was immobilized on glass substrate and these were characterized by SEM images. Fig. 4a and b show the surface morphology before and after enzyme immobilization respectively. It clearly shows that by the covalent binding technique the enzyme is successfully immobilized on a glass surface with uniformity. Uniformity and quality of enzyme (bio-molecule) coverage on the fiber surface is important for successful working of the sensor. Prior to enzyme immobilization sensor probe showed resonance wavelength of 1544.8 nm which reduced to 1540.9 nm after the enzyme immobilization. The change in resonance wavelength of LPG can be correlated to the addition of the higher index bio-recognition layer on the cladding surface as shown in Fig. 4b. The addition of a higher index layer results in the increase in the value of the effective index of the cladding mode, $n_{cl}^{(m)}$ and hence the resonance wavelength is reduced (Eq. (2)). In addition, enzyme immobilization on glass substrates was also confirmed using Raman spectroscopy (Rainishaw Invia) with an Argon laser source (excited at 514 nm). The peaks in the Raman spectrum of immobilized lipase enzyme on the glass substrates shown in Fig. 4c. (ii), (iii) and (iv) match the peaks of native lipase enzyme shown in Fig. 4c. (i). However, there is an extra peak at 1603 cm^{-1} in sample (iii) which may be due to presence of some impurity.

Further, the stability of the enzyme immobilization on the glass fiber was confirmed by lipase assay by observing the optical density (O.D.) of reaction by spectrophotometric analysis using p-nitrophenyl palmitate (Wrinkler and Stuckman, 1979). Insignificant leaching of enzyme was observed after number of trials as shown in Fig. 4d. This observation verified that the main properties of native lipase enzyme are conserved after immobilization on the fiber surface and lipase remains firmly attached to the fiber surface even after a number of uses.

To study the response of enzyme immobilized LPG, the sensor was exposed to different concentrations of triacylglyceride emulsion. Emulsions of varying concentrations of triacylglyceride ranging from 0 mM to 7 mM were poured into the flow cell one by one and the spectrum was observed and recorded after 1 minute stabilization time on the OSA. After each recording of the spectrum, the analyte was dipped out and probe was rinsed copiously with PBS, until the initial spectrum was obtained. The flow cell was kept at a constant temperature 37 °C throughout the experiment using the feedback temperature control unit.

Fig. 5a shows the transmission spectrum recorded at different concentrations of triacylglyceride. It can be seen that the transmission spectrum changes with the concentrations of

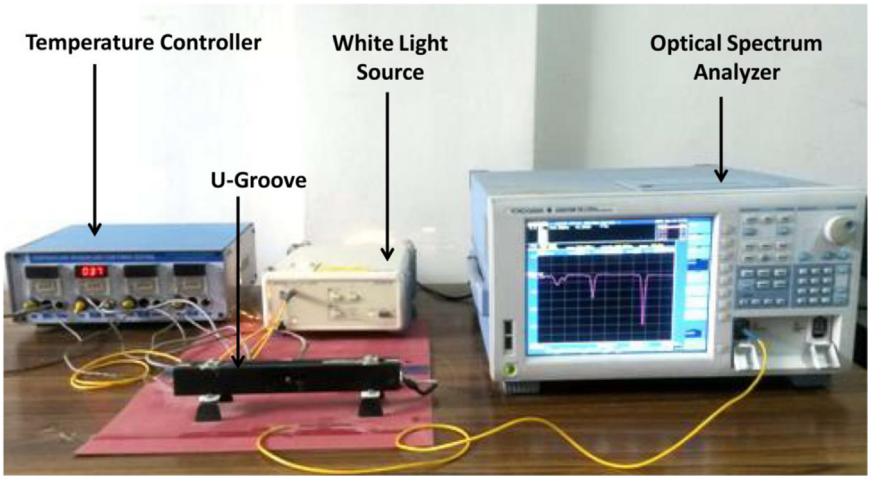


Fig. 3. Photograph of the experimental setup for the detection of triacylglycerides.

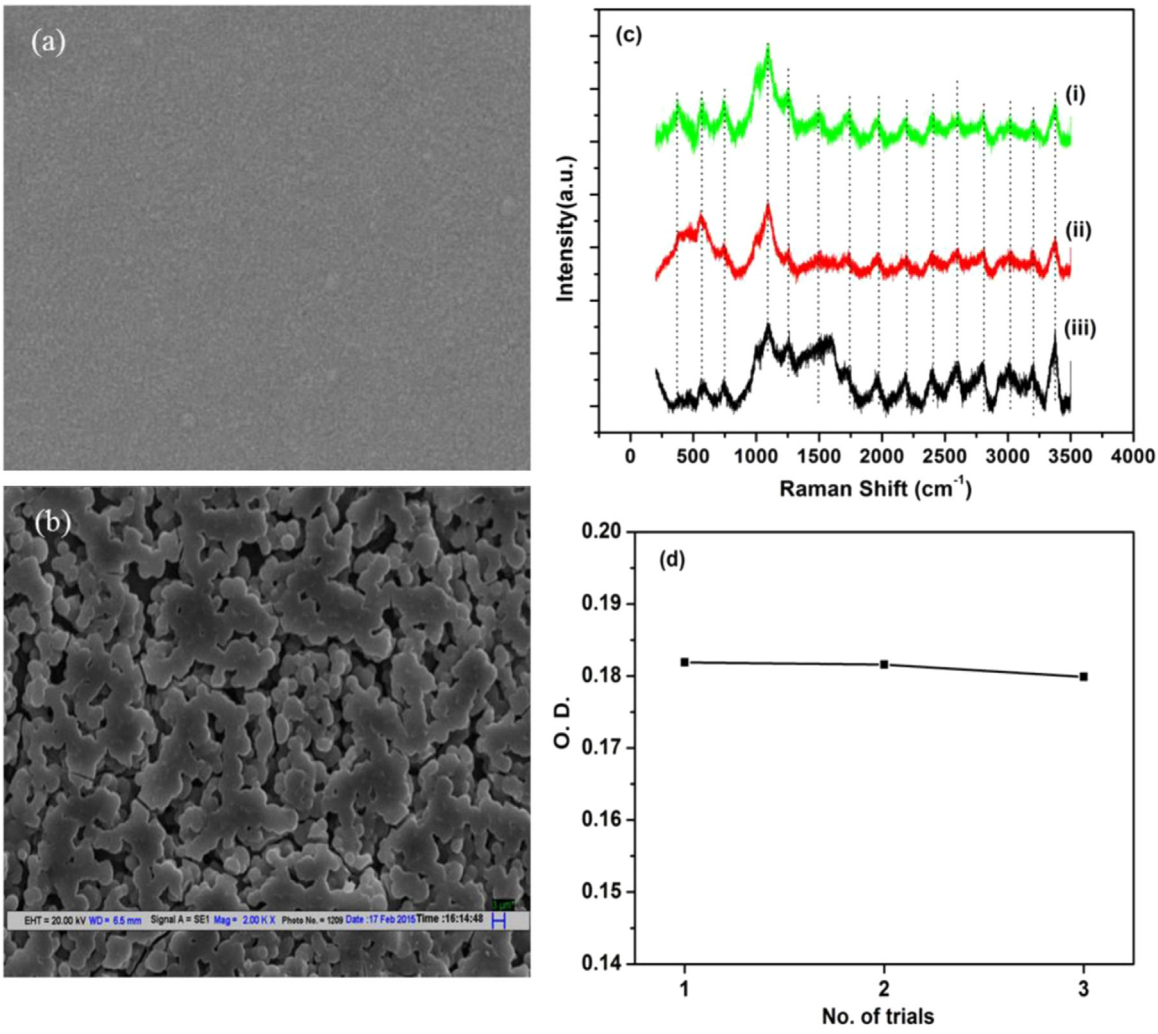


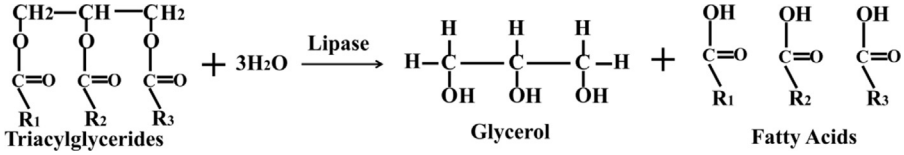
Fig. 4. (a) Scanning electron microscope image of the surface of glass substrate before immobilization of enzyme (b) Image of the surface after immobilization of enzyme (c) Raman spectral studies of native lipase enzyme (i) and lipase immobilized substrates (ii), (iii) and (iv) (d) Variation in optical density with no. of trials.

triacylglyceride and the resonance dips shift towards lower wavelengths as the concentration increases. A total of 3.5 nm change in resonance wavelength was observed for triacylglyceride concentrations varying from 0 mM to 7 mM. The shift in resonance wavelength along with corresponding error bar at each point as a function of triacylglyceride concentrations are shown in the Fig. 5b. While drawing error bar, measurement accuracy of the optical spectrum analyzer and volume measuring device were also taken into consideration. The symbols are experimental points while the solid line is a polynomial fit to experimental data.

To understand the occurrence of a shift in resonance wavelength, it is important to take a look at the interaction of the triacylglycerides molecules with lipase enzyme at the interface of the bio-recognition layer as discussed by Jurado et al. (2006) and Hermansyah et al. (2006). The complete hydrolysis of the triacylglycerides molecules by lipase enzyme involves a three step process as discussed by Hermansyah et al. (2006). The lipase enzyme reacts with the adsorbed triacylglycerides to form an enzyme-substrate complex (ET). The lipase enzyme in the presence of water hydrolyses each of the ester bonds to release diacylglycerides, monoacylglycerides, glycerol and fatty acids in sequence as shown in Eq. (3). The final products are shown in Eq. (4).

$$\begin{aligned} E + T &\rightarrow ET \\ T + W &\rightarrow D + F \\ D + W &\rightarrow M + F \\ M + W &\rightarrow G + F \end{aligned} \tag{3}$$

Here E, T, W, D, F, M and G denote lipase enzyme, triacylglycerides, water, diacylglycerides, fatty acid, monoacylglycerides and glycerol, respectively.



(4)

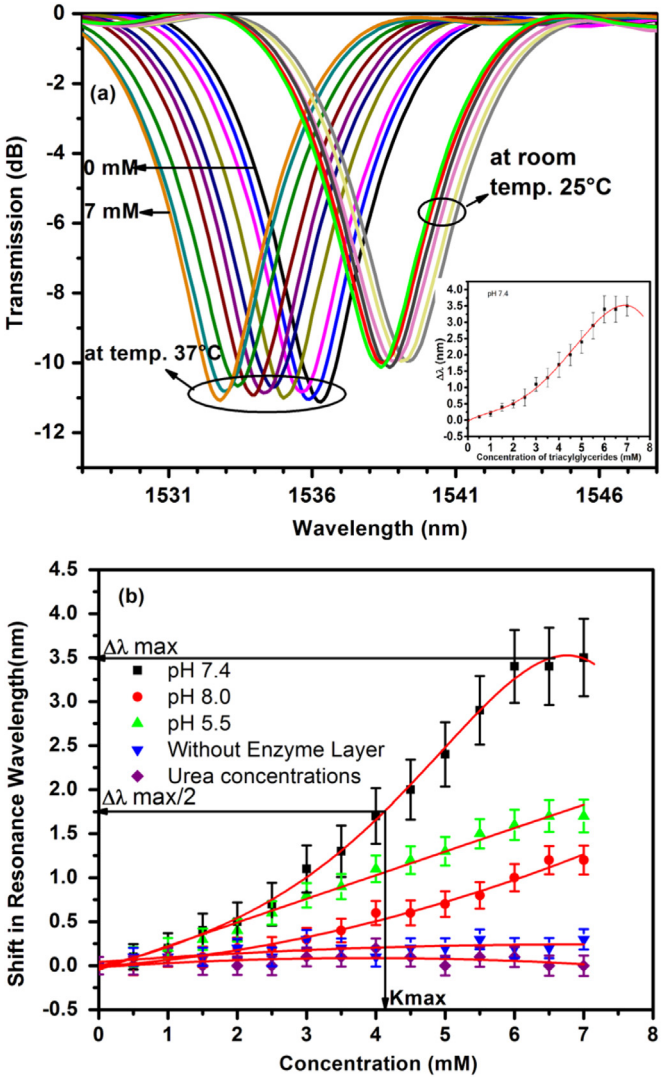


Fig. 5. (a) Transmission spectrum at different concentrations of triacylglyceride (TGs) at room temperature and 37 °C, corresponding shift in resonance wavelength vs. concentrations of triacylglyceride along with standard deviation in the inset (b) corresponding shift in resonance wavelength vs. concentrations of triacylglyceride ranging from 0 mM to 7 mM for pH 5.5, pH 7.4 and pH 8.0. Also plotted are the shifts in resonance wavelength without enzyme layer and with urea analyte with concentrations ranging from 0 mM to 7 mM.

However, in the case of the immobilized enzyme on the fiber, the hydrolysis of the triacylglycerides molecule would require direct contact between the interfacial enzyme layer and the ester bonds in the molecule. Due to the tetrahedral arrangement of carbon, only one ester group will be in contact with lipase active site and when hydrolysis occurs, diacylglycerides will be formed along with the fatty acid. The accumulation of diacylglycerides and fatty acid at the interface inhibits further hydrolysis of the triacylglycerides molecules. The rate of reaction depends on the concentration of triacylglycerides molecules at the interface, which is proportional to the concentration in the analyte. The adsorption of the triacylglycerides molecules and accumulation of diacylglycerides and fatty acids results in the effective increase in the thickness and refractive index of the bio-recognition layer. Any change in the cladding ambient interface changes the effective index of the cladding mode ($n_{cl}^{(m)}$) and consequently the resonance wavelength also changes according to Eq. (2). The theoretical model in Section 4 shows how an increase in refractive index and thickness results in the shift of resonance towards shorter wavelength.

As a control experiment, the LPG without the bio-recognition enzyme layer was also immersed in triacylglyceride emulsions of different concentrations. Negligible change in resonance wavelength was observed as shown in Fig. 5b which confirms that there is no change in refractive index of the bulk analyte for different concentrations of triacylglyceride.

The pH of the buffer is a major factor which is known to influence the enzyme activity. To study this, we repeated the experiment with phosphate buffer solutions of pH 5.5 and 8.0. The corresponding transmission spectrum show smaller shift in the resonance wavelength in comparison to the shift at pH 7.4. Comparison of the shift in resonance wavelength with concentrations of triacylglyceride at different pH of phosphate buffer solution is shown in Fig. 5b. The sensor has maximum sensitivity of 0.5 nm/mM at pH 7.4 in comparison to the sensitivity of 0.171 nm/mM and 0.228 nm/mM at a pH of 5.4 and 8.0 respectively.

The sensor probe was also prepared by immobilizing lipase using different concentrations of enzyme solution varying from 2 mg/ml to 6 mg/ml. It was observed that sensor shows maximum response when immobilization was carried out by use of a 4 mg/ml solution of lipase. For lower concentrations, there is insufficient immobilized enzyme on the LPG surface to hydrolyses the triacylglycerides. Sensitivity of the sensor decreases with increase in the concentration of enzyme beyond 4 mg/dl. This may have been caused by multilayer formation (aggregation) of enzyme, as well as the structural deformation of active sites of the immobilized enzymes.

Further, the Sensor probe was replicated five times for the same concentration of lipase and same immobilization steps. The shift in resonance wavelength as a function of triacylglyceride concentrations in each case are shown in the inset of Fig. 5a along with corresponding standard deviation at each point. Maximum standard deviation value of 10% was observed, implying that the sensor probe is highly reproducible.

We also estimated the Michaelis constant (K_m) for the reaction between lipase and triacylglycerides from the plot between concentration of triacylglycerides and shift in the resonance wavelength at pH 7.4 as shown in Fig. 5b. K_m is defined as the substrate concentration at ½ the maximum velocity of reaction, which in this case is measured as shift in resonance wavelength. From the results shows in Fig. 5b, K_m of this sensor is obtained 4.20 mM, which is higher than the earlier reported values of 1.14 mM (Dhand et al., 2010), 2.51 mM (Lanlan et al., 2015) and 0.12 mM (Wu et al., 2014). The high K_m value implies that this sensor can be used for measuring a wide range of the triacylglyceride concentrations.

Experiment was also carried out for specificity of target analyte (triacylglycerides), as there are many other interfering substances present in blood such as urea, glucose and ethanol etc. The sensor was exposed to solutions with different concentration of urea in the physiological range of urea in human blood, i.e., from 0 mM to 7 mM (6–42 mg/dl). 1 mM of urea concentration is equivalent to 6 mg/dl urea. It is observed that negligible shift in resonance wavelength occurs when sensor probe exposed to different concentrations of urea analyte as shown in Fig. 5b. This confirms that sensor is highly specific to the triacylglycerides detection. To study the interference effect of other substances on the detection of triacylglycerides at physiological conditions, we added glucose, urea and ethanol at their physiological levels of 150 mg/dl, 42 mg/dl and 80 mg/dl respectively into the triacylglyceride solutions. No change was observed in the response curve of the sensor. This study confirmed that the presence of other substances has negligible effect on the detection of triacylglycerides.

The effect of time on sensor response was also studied by observing the transmission spectrum from 10 s to 1 h. After 1 min, no change in resonance wavelength was observed. The biosensor

response was also measured at room temperature (25 °C). It is observed that there is a very small shift in resonance wavelength as shown in Fig. 5a. Hence, it can be concluded that the sensor response has a high sensitivity at temperature 37 °C (normal human body temperature), which is in good agreement with the published literature on lipase activity (Kumari et al., 2012).

We have also evaluated the performance of the sensor in terms of detection accuracy. Detection accuracy is defined as how accurately a sensor can detect the resonance wavelength and it depends on the width of the transmission curve around its minima. Different authors use different definitions of detection accuracy. Here, we have defined detection accuracy as $1/\delta\lambda$, where $\delta\lambda$ is the width of transmission curve corresponding to 1 dB above the minima of the transmission curve. It is found that detection accuracy for a particular value of pH is nearly equal in the full range of the analyte concentration and is maximum for pH value of 7.4. It may also be noted that limit of detection of the sensor is 17.71 mg/dl (0.2 mM) when the resolution of the optical spectrum analyzer is 0.1 nm this value is lower than those reported earlier: 0.35 mM (Bhambi et al., 2006), 0.28 mM (Narang et al., 2010b), 50 mg/dl (Liao et al., 2008) and 0.42 mM (Reddy et al., 2001). If we use 0.01 nm as resolution of the optical spectrum analyzer the limit of detection of sensor can be improved to 1.77 mg/dl.

4. Modeling of the LPG bio-sensor

To understand the response of the LPG sensor theoretically, we used a four layer model for the long period fiber grating. Fig. 1a shows the refractive index profile of the high index bio-recognition layer coated fiber, where, n_1 is the refractive index of the fiber core, n_2 is the refractive index of the pure silica fiber cladding, n_3 is the refractive index of the few nanometer thick high index bio-recognition layer and n_4 is the ambient refractive index. For the fiber used in fabrication of LPG the numerical aperture is 0.12 with diameters of core and cladding as 8.2 μm and 125 μm respectively. Refractive index of high index bio-recognition layer is assumed to be $n_3=1.5$ with a thickness d_3 of 280 nm. Refractive index of surrounding analyte is taken to be the measured value, $n_4=1.333$. The long period grating formed in the core has a period of 581 μm, length of 6 cm and peak index modulation Δn of 7×10^{-5}.

First, we studied the effect of refractive index of analyte on the transmission spectrum of LPG with the bio-recognition layer keeping $n_3=1.5$ and $d_3=280$ nm. It is observed that negligible change in the transmission spectrum occurred due to the variation in the refractive index of analyte (n_4) from 1.333 to 1.340, confirming that the response in the transmission spectrum or basic principle of the sensor is not change in refractive index of the ambient analyte.

Hence, to model the effect of analyte on the bio-recognition layer and the transmission spectrum, we considered both possible changes, i.e., the change in the refractive index and change in thickness of the high index bio-recognition layer. First the refractive index of bio-recognition layer was varied from $n_3=1.50$ to $n_3=1.51$ with $d_3=280$ nm and $n_4=1.333$. The corresponding transmission spectrum of LPG shown in Fig. 6a show that resonance wavelength shifts towards shorter wavelengths. Similarly, the thickness d_3 was varied from 280 nm to 320 nm keeping $n_3=1.5$ and $n_4=1.333$. The transmission spectrum shown in Fig. 6b again show that the resonance wavelength shifts towards the shorter wavelengths.

Finally, the effect of both, change in refractive index and thickness of bio-recognition layer simultaneously, on the response was studied as shown in Fig. 6c. The shift in resonance wavelength due to the change in thickness from 280 nm to 315 nm and

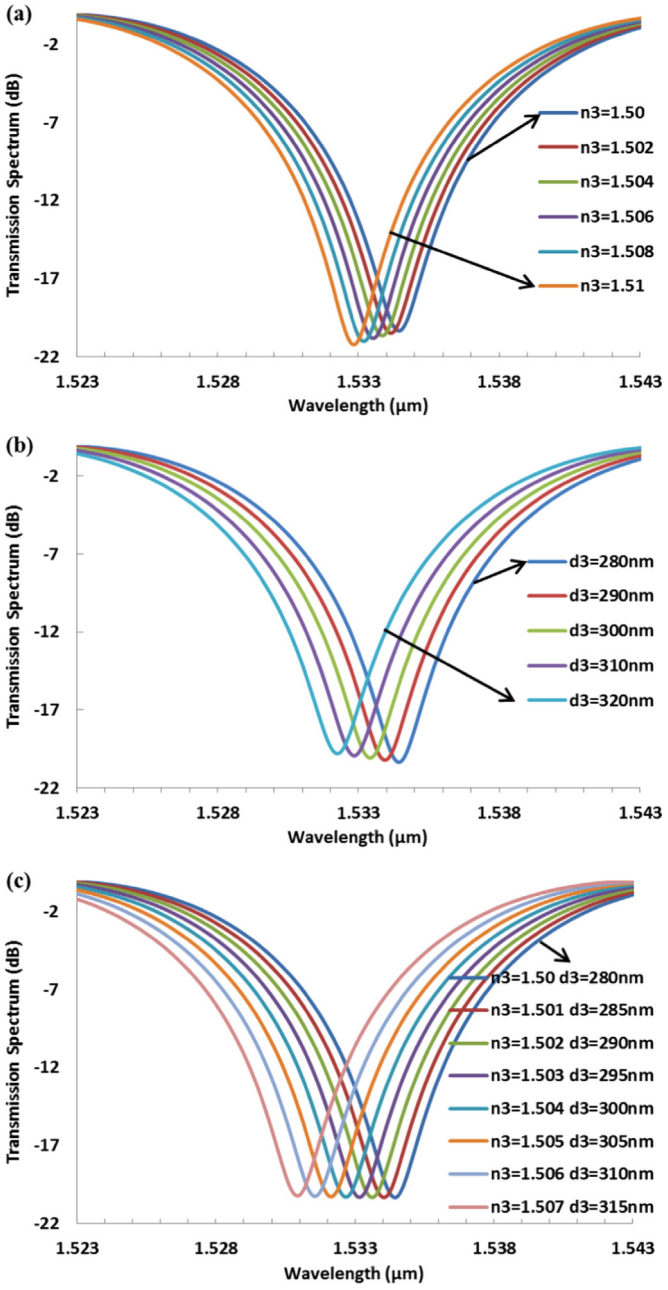


Fig. 6. (a) Transission spectrum changes for $n_3=1.50$, 1.502, 1.504, 1.506, 1.508 and 1.510, with $d_3=280$ thick high index bio-recognition layer and $n_4=1.333$ (b) Transission spectrum changes with thickness of high index layer $d_3=280$, 290, 300, 310, 320 and 330 nm with $n_3=1.5$ and $n_4=1.333$ (c) Transission spectrum with varying both thickness $d_3=280$–315 nm and refractive index $n_3=1.50$–1.507 of the high index bio-recognition layer with $n_4=1.333$.

refractive index from 1.50 to 1.507 of bio-recognition layer is comparable with shift obtained experimentally. Hence, we can conclude that the experimental results obtained are best modeled by assuming a change in both, thickness and refractive index of the bio-recognition layer on interaction with the triacylglycerides emulsion as discussed in Section 3.1.

5. Conclusions

In the present work we have developed a long period fiber grating based sensor for the label free quantitative estimation of triacylglyceride concentration by the successful covalent

immobilization of lipase enzyme directly on the optical fiber. Immobilization has been confirmed by the Raman Spectroscopy and Scanning Electron Microscope images. The stability of the immobilized enzyme on the fiber was also confirmed by lipase p-nitrophenyl palmitate assay by observing the optical density (O. D.) of the reaction using spectrophotometric analysis. The shift in resonance wavelength of the transmission spectrum of the LPG coated with a layer of lipase enzyme can be calibrated to obtain the concentrations of triacylglyceride. A high sensitivity of 0.5 nm/mM for a pH 7.4 phosphate buffer solution at temperature of 37 °C is observed. Sensitivity can be improved by choice of a lower grating period of the LPG corresponding to a higher cladding mode which is more sensitive to change in ambient conditions. Response time of the sensor is around one minute and limit of detection is 17.71 mg/dl when the resolution of the optical spectrum analyzer is 0.1 nm. The LPG with a bio-recognition layer is also modeled theoretically. The results obtained experimentally have been shown to be best modeled by assuming a change in both the thickness and refractive index of high index bio-recognition layer simultaneously when the enzyme layer interacts with the triacylglyceride emulsion. The various advantages of the present sensor include high sensitivity, less response time, label free detection, ease of fabrication, low detection limit, good stability and high selectivity.

Acknowledgment

The financial support from University Grants Commission, India and University of Delhi research grant, India is greatfully acknowledged.

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