A fiber optic biosensor for the detection of cholesterol levels based on chitosan coated long period grating

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A fiber optic sensor for the measurement of total cholesterol is designed and developed. The developed chitosan coated long period grating (LPG) sensor shows a sensitivity of 5.025×10^6 pm·mL/g in the measurement range of the sensor. The sensor also shows a linear response in the measured range of cholesterol levels, which is highly desirable for exploitation as a commercial cholesterol sensor.

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In the last three decades, many studies have revealed the relationship between the increased cholesterol concentration and the occurrence of cardiovascular diseases, like arteriosclerosis and hypertension. Hence, the detection and control of cholesterol have become highly significant in quality control of food products.

Many procedures, such as fluorescence detection[1,2], electrophoresis[3], Raman spectroscopy[4], high-performance liquid chromatography (HPLC)[5], gas-liquid chromatography[5,6] and using enzymes[7], have been reported earlier for the detection and estimation of cholesterol. However, the majority of these methods do not assure on-site monitoring of cholesterol. Even though enzymatic procedures ensure the specificity and selectivity required for these kinds of assays, the use of enzymes makes the fabrication and handling of the sensor head difficult and costly. Hence, the development of simple, inexpensive, direct and real-time cholesterol sensors is of continuing interest, because the traditional methods for cholesterol measurement require costlier laboratory analyses.

Optical fiber long period gratings (LPGs) are being used extensively in sensing applications for the last few decades. In this paper, we propose a cholesterol biosensor exploiting the sensitivity of chitosan coated LPGs to test the concentration of the cholesterol sample solution.

An LPG operates by the coupling of the fundamental core mode (i.e., the LP_{01} mode) to the co-propagating cladding modes (LP_{0m} mode with m= 2, 3, 4 ...) in the fiber. This coupling of power results in the formation of rejection bands around specific wavelengths (resonant wavelengths) in the transmission spectrum of the LPG. These resonant wavelengths are given by the phase-matching equation as

$$\lambda_m = (n_{\text{eff}}^{\text{co}} - n_{\text{eff},m}^{\text{cl}})\Lambda, \tag{1}$$

where λ_m is the resonant wavelength corresponding to the coupling to the mth cladding mode, Λ is the grating period, $n_{\text{eff}}^{\text{co}}$ is the effective refractive index of the fundamental core mode (LP_{01}), and $n_{\text{eff},m}^{\text{cl}}$ is the effective refractive index of the mth order cladding mode (LP_{0m}). External perturbations, like strain, temperature, bending and surrounding refractive index (SRI)[8], affect the coupling strength between the core and cladding modes, which leads to the amplitude changes as well as the wavelength shift of the resonant peaks in the LPG transmission spectrum.

The operation of the LPG based chemical sensor is based on the refractive index sensitivity of the LPG due to the dependence of the effective index of the cladding mode $n_{\text{eff},m}^{\text{cl}}$ on the SRI. The effect of SRI on the resonant wavelength[9] is determined by

$$\left(\frac{\mathrm{d}\lambda}{\mathrm{d}n_{\text{s}}}\right)_m = \left(\frac{\mathrm{d}\lambda}{\mathrm{d}n_{\text{eff},m}^{\text{cl}}}\right)\left(\frac{\mathrm{d}n_{\text{eff},m}^{\text{cl}}}{\mathrm{d}n_{\text{s}}}\right), \tag{2}$$

where $\mathrm{d}n_{\text{s}}$ is the change in SRI. It is known that the sensitivity of LPG to the SRI can be enhanced by providing one or more layers of coatings of reactive materials over the grating region[10,11].

In order to enhance the sensitivity of cholesterol sensing, a thin layer of chitosan was coated over the

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LPG. Chitosan is a polysaccharide obtained by deacetylisation of chitin, which is the major constituent of the exoskeleton of crustaceous water animals[12]. Chitosan can selectively bind materials, such as cholesterol, fat, metal ion and protein[13]. Chitosan contains three types of reactive functional groups, which are an amino/acetamido group as well as a primary and a secondary hydroxyl groups. As the sample of cholesterol is introduced, the cholesterol gets attached to these active sites on the layer of coating. The combined effects of electrostatic attraction, embedding, adsorption and entrapment are the probable mechanisms for the cholesterol binding effects of chitosan[14]. This binding of cholesterol in turn enhances the sensitivity of LPG to the SRI, and this principle is used in the realization of the cholesterol sensor.

LPG with a grating period of 435 μm was fabricated by KrF excimer laser source (248 nm) through point-by-point writing method on SMF-28 (SMF-28e, Corning) fiber.

The sensor head was fabricated by coating the LPG with a thin layer of chitosan. 0.25 mg of high molecular weight chitosan powder with degree of deacetylisation of 98% was stirred well with 1 mol acetic acid at room temperature for 5 h to get a clear solution. Dip coating technique was used to coat the LPG with chitosan. The coated fiber was dried in air at room temperature to avoid cracks.

The scanning electron microscope (SEM) image of the coating shown in Fig.1(a) depicts a uniform surface layer of chitosan without any cracks. Fig.1(b) shows the end view of the fiber with coating. The chitosan coating on the fabricated sensor head has a thickness of 1.461 μm.

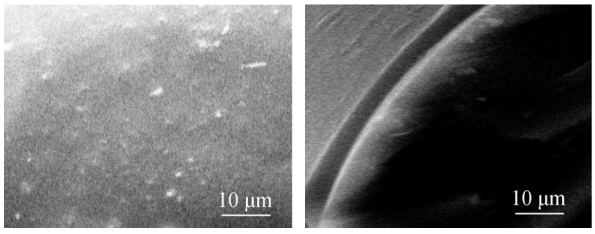

Fig.1 SEM images of (a) the surface of chitosan coated layer and (b) the end view of the fiber with coating

The chitosan coated LPG sensor head was fixed in a specially designed glass cell with epoxy as shown in Fig.2. Provisions for filling the sample and draining it out as and when desired were provided in the cell. The transmission spectrum of the LPG was studied with an optical spectrum analyzer (OSA) (Yokogawa-AQ6319) and a white-light source (Yokogawa-AQ4305). Accurate measurements were ensured by maintaining the temperature of the experimental setup and sample solution at 25.0 °C±0.5 °C. In addition, the test sample with a volume of 30 mL was used, so that the fiber section containing LPG was immersed completely in test sample throughout the experiments. The humidity around the test setup was also monitored to avoid the influence of humidity on the coating.

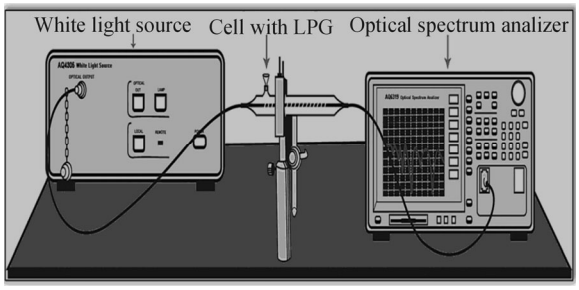

Fig.2 Schematic diagram of experimental setup

At the end of each measurement, the glass cell and the LPG sensor head were cleaned with distilled water and isopropyl alcohol repeatedly, followed by proper drying, so that the initial transmission spectrum of LPG in air was obtained.

Pure cholesterol ($C_{27}H_{46}O$) purchased from Sigma Aldrich was used for preparing the sample solutions with different cholesterol concentrations ranging from 0 g/mL to 5×10^{-3} g/mL, by dissolving definite amount of cholesterol in coconut oil with refractive index of 1.448. The refractive indices of these sample solutions were found to vary from 1.448 to 1.455.

Fig.3 shows the transmission spectra of LPG with a period of 435 μm in air with and without the coating of the chitosan layer, and it also depicts the transmission spectrum of the coated LPG when it was immersed in pure coconut oil. For the used LPG, the maximum power coupling to the cladding mode was observed corresponding to the resonant peak of LP_{04} mode at 1 568.93 nm in air. This resonant peak exhibits the maximum response at the test conditions, compared with other resonant modes.

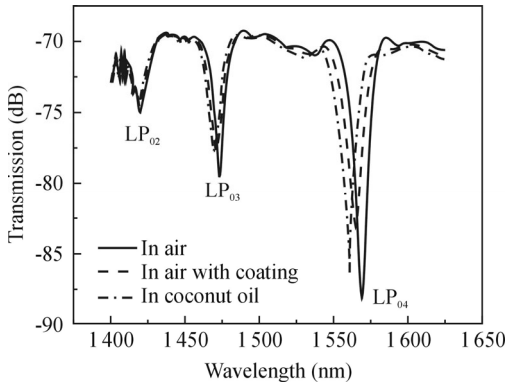

Fig.3 Transmission spectra of LPG in air (with and without coating) and in solvent (with coating)

The resonant peak of LP_{04} mode at 1 568.93 nm in air was shifted to 1 565.37 nm after the prepared coat-

ing was dried. When pure solvent is introduced into the glass cell, the resonant peak of LP_{04} shows a remarkable blue shift. The loss peak of LP_{04} mode has a blue shift from 1 565.37 nm to 1 560.87 nm as the surrounding medium is altered from air to coconut oil. Along with the blue shift in the wavelength, the resonant peak amplitude is increased from −83.18 dB to −86.374 dB. Hence, further investigations will be centered on the LP_{04} mode of the transmission spectra in the wavelength range 1 520 nm to 1 580 nm.

Fig.4 shows the transmission spectra of chitosan coated LPG sensor head with various cholesterol concentrations in coconut oil. When the concentration of the test solutions is increased, a blue shift of the LP_{04} resonant peak is observed. The LPG exhibits a total blue shift of approximately 25.12 nm when the concentration of cholesterol is changed up to 5×10^{-3} g/mL. This spectral shift of 25.12 nm, noticed for a refractive index range of the sample solutions from 1.448 to 1.455, corresponds to an average resolution of 2.78×10^{-4} nm^{-1}.

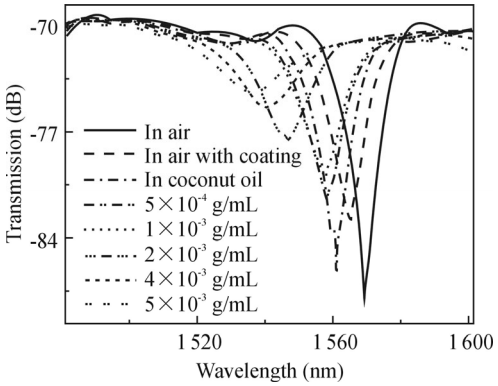

Fig.4 Transmission spectra of chitosan coated LPG with different concentrations of cholesterol

The sensitivity of the LPG is shown in Fig.5, when it is used as a sensor for various concentrations of cholesterol dissolved in coconut oil.

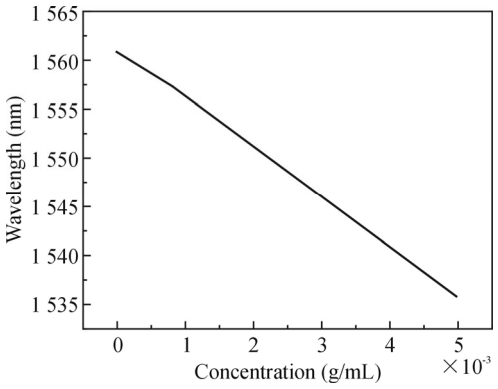

Fig.5 Resonant wavelength (LP_{04}) peak positions as a function concentration of cholesterol

The overall sensitivity in the measurement range of the sensor is around 5.025×10^{6} pm·mL/g of cholesterol, which is more than double of that of the uncoated LPG sensor which was reported earlier[15]. Throughout the range of measurement, the sensor shows a linear response, which is highly appreciable for a commercial cholesterol sensor.

The transmitted intensity of the resonant wavelength (LP_{04}) with respect to the different concentrations of cholesterol in the measurement range is shown in Fig.6.

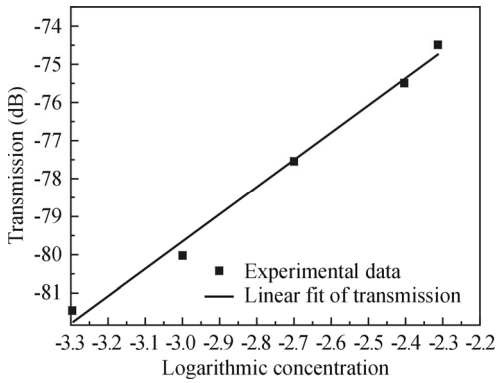

Fig.6 Transmitted intensities at resonant wavelength (LP_{04}) peak as a function of logarithmic concentration of cholesterol

As the cholesterol concentration increases, the SRI increases to approaching the cladding refractive index of the fiber, which can reduce the coupling between the core and cladding modes. This reduced coupling is attributed to the reduction in the amplitude of the LP_{04} loss peak. In this experiment, the amplitude of the LP_{04} resonant wavelength peak is decreased from −81.48 dB to −74.46 dB as the concentration of cholesterol in coconut oil is varied up to 5×10^{-3} g/mL. A linear response of the transmitted intensity is also observed in the measurement range of cholesterol concentration. This intensity modulation can also be utilized along with the wavelength coded information to have better results for a commercial sensor.

The results shown in this paper depict the application of fiber optic LPG based system for the sensing and measurement of cholesterol concentration. The wavelength as well as the intensity modulation characteristics can be utilized in designing cholesterol sensors for commercial applications. The sensor presented here provides a real time response and requires only a small volume of the sample for analysis.

Added features of the sensor, like simplicity and high sensitivity, make it recommendable for medical diagnosis and clinical applications for the detection of cholesterol concentration in humans with suitable modifications. The system can be effectively employed in the areas of chemical and biomedical sensing, drug development, etc. The wide range and linear response are the other attractive features of the developed sensor.

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