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

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A Fiber Optic sensor for the measurement of total cholesterol has been designed and developed. The chitosan coated Long Period Grating (LPG) sensor developed showed a sensitivity of 5.025pm/10^{-6}g/ml in the measurement range of the sensor. The sensor also showed a linear response in the measured range of cholesterol levels, which is highly desirable for exploitation as a commercial cholesterol sensor.

Cholesterol is a vital lipid present in practically all cells of mammals, and is highly essential for its functioning. They also form the basic components of the nerve and brain cells [1] and are the precursors for biological materials, such as bile acid and hormones [2]. The cholesterol concentration in the blood of a healthy, human being is in the range of 1400 to 2000×10^{-6}g/ml [3]. An excess of cholesterol can lead to major health related problems in living beings.

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

Many procedures, such as, fluorescence detection [4, 5], electrophoresis [6], Raman spectroscopy [7], HPLC [8], gas-liquid chromatography [8, 9], using enzymes [10] etc. have been reported earlier for the detection and estimation of cholesterol. However, the majority of these methods does 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, development of simple, inexpensive, direct and real-time cholesterol sensors is of continuing interest, as the traditional methods for cholesterol measurement require costlier laboratory analyses.

Optical Fiber Long Period Gratings (LPG) 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 the concentration of the cholesterol sample solution under test.

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 (1):

$$\lambda_m = [n_{eff}^{co} - n_{eff,m}^{cl}]\Lambda \qquad (1)$$

where λ_m is the resonant wavelength corresponding to coupling to the m^{th} cladding mode, Λ is the grating period, n_{eff}^{co} is the effective refractive index of the fundamental core mode (LP_{01}), $n_{eff,m}^{cl}$ is the effective refractive index of the m^{th} order cladding mode (LP_{0m}). External perturbations like strain, temperature, bending and surrounding refractive index (SRI) [11] affect the coupling strength between the core and cladding modes, which leads to amplitude changes as well as wavelength shift of the resonant peaks in the LPG transmission spectrum.

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 ($n_{eff,m}^{cl}$) on the SRI. The effect of refractive index of the surrounding medium on the resonant wavelength [12] is determined by equation. (2).

$$\left(\frac{d\lambda}{dn_s}\right)_m = \left(\frac{d\lambda}{dn_{cl,m}^{eff}}\right)\left(\frac{dn_{cl,m}^{eff}}{dn_s}\right) \qquad (2)$$

where dn_s is the change in the refractive index of the surrounding material. 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 [13, 14].

In order to enhance the sensitivity of cholesterol sensing, a thin layer of chitosan was coated over the LPG. Chitosan is a polysaccharide obtained by deacetylisation of chitin, which is the major constituent of the exoskeleton of crustaceous water animals [15]. Chitosan can selectively bind materials such as cholesterol,

fats, metal ions, proteins etc. [16]. Chitosan contains three types of reactive functional groups, an amino/acetamido group as well as a primary and a secondary hydroxyl group. 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. [17]. 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 435µm was fabricated with KrF - Excimer laser source (248nm), 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. 250mg of high molecular weight chitosan powder with 98% degree of deacetylisation was stirred well with 1 molar acetic acid at room temperature for 5 hours 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.

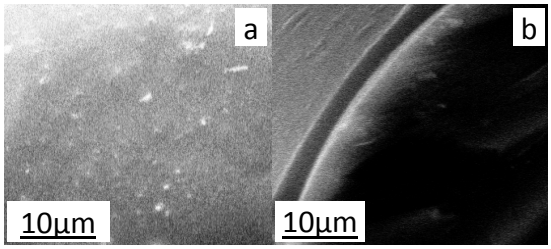

Fig: 1. SEM images of the chitosan coated layer.

The 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 had a thickness of 1.461µm.

The chitosan coated LPG sensor head was fixed in a specially designed glass cell with epoxy as shown in fig.2. Provision 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 - AQ 4305). Accurate measurements were ensured by maintaining the temperature of the experimental setup and sample solution at 25.0 ± 0.5°C. In addition, a volume of 30ml of the test sample was used, so that the fiber section containing LPG was immersed completely in samples throughout the experiments. The humidity around the test setup was also monitored to avoid the influence of humidity on the coating.

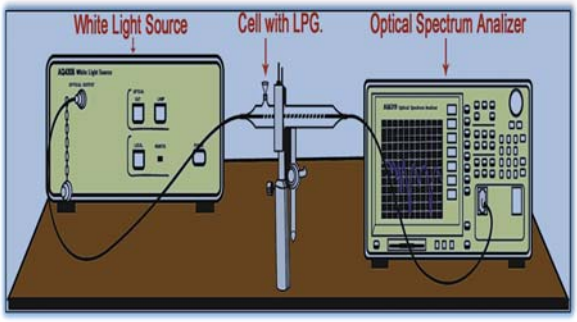

Fig: 2. Experimental Setup.

At the end of each measurement, the glass cell and the LPG sensor head were cleaned with distilled water and then with 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 of varying concentrations ranging from 0 to 5000 $\times 10^{-6}$g/ml of cholesterol, by dissolving definite amount of cholesterol in coconut oil having a 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 435µm in air, with and without the coating of the chitosan layer.

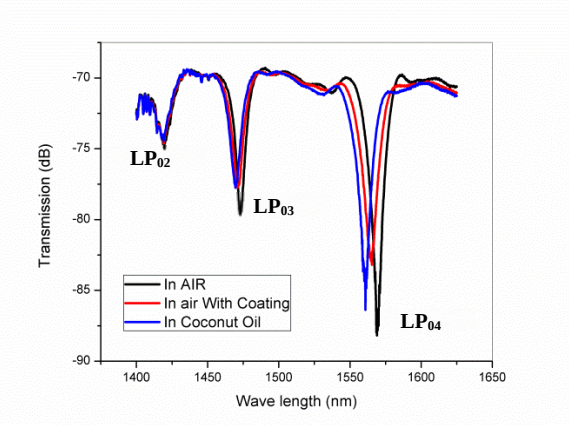

Fig: 3 Transmission spectra of LPG in air (with and without coating)and in solvent (with coating).

Fig.3 also depicts the response of the coated LPG when it was immersed in pure coconut oil. For the LPG used, maximum power coupling to the cladding mode was observed corresponding to the resonant peak of LP_{04} mode at 1568.93nm in air. This resonant peak exhibited maximum response to the test conditions, compared to other resonant modes.

The resonant peak of LP_{04} mode at 1568.93nm in air, was shifted to 1565.37nm after the prepared coating was dried. When pure

solvent was introduced into the glass cell the resonant peak of LP_{04} showed a remarkable blue shift. The loss peak of LP_{04} mode had a blue shift from 1565.37nm to 1560.87nm as the surrounding medium was altered from air to coconut oil. Along with the blue shift in the wavelength, the resonant peak amplitude increased from -83.18dB to -86.374dB. Hence, further investigations were centered on the LP_{04} mode of the transmission spectra in the wavelength range 1520nm to 1580nm.

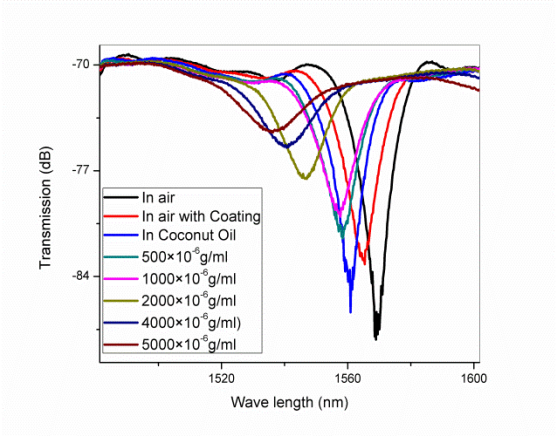

Fig: 4 Transmission spectra of chitosan coated LPG for different levels of cholesterol

Fig: 4 shows the transmission spectra of chitosan coated LPG sensor head for various concentrations of cholesterol in coconut oil. When the concentration of the test solutions was increased, a blue shift was observed in the LP_{04} resonant peak wavelength. The LPG exhibited a total blue shift of approximately 25.12nm when the level of cholesterol was changed up to 5000 $\times 10^{-6}$g/ml. This spectral shift of 25.12nm, noticed for a refractive index range of 1.448 to 1.455 of the sample solutions, corresponds to an average resolution of 2.78×10^{-4} nm^{-1}.

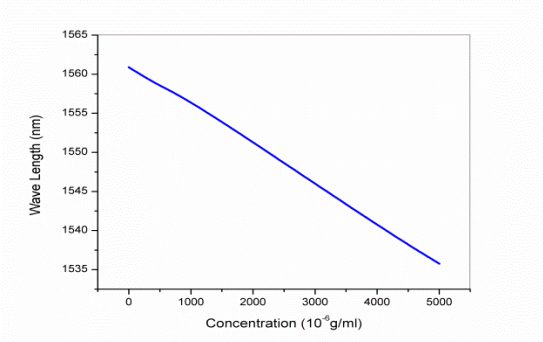

Fig. 5 Resonant Wavelength (LP_{04}) Peak positions as a function of different levels of cholesterol

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

The overall sensitivity in the measurement range was around 5.025pm/10^{-6}g/ml of cholesterol, which is more than double that of the uncoated LPG sensor, reported earlier [18]. Throughout the range of measurement, the sensor showed 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 levels of cholesterol, in the measured range is shown in Fig. 6.

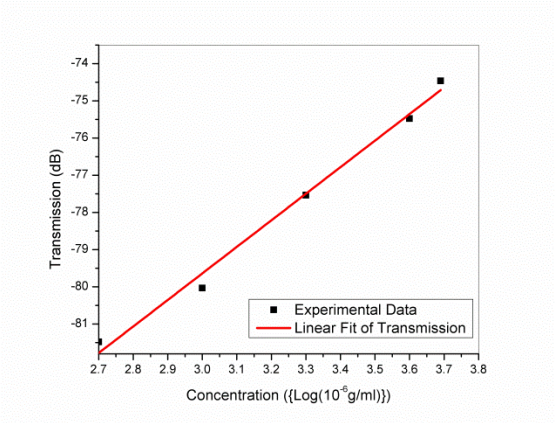

Fig. 6 Transmitted intensities of Resonant Wavelength (LP_{04}) Peak as a function of different levels of cholesterol

As the cholesterol concentration increases, the SRI increases, and approaches the cladding refractive index of the fiber, reducing 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 decreased from -81.48dB to -74.46dB as the concentration of cholesterol in coconut oil was varied upto 5000 $\times 10^{-6}$g/ml. A linear response of the transmitted intensity was also observed in the measured range of cholesterol levels. This intensity modulation can also be utilized along with the wavelength coded information to have better results for a commercial sensor.

The results showed in this work depict the application of fiber optic LPG based system for the sensing and measurement of cholesterol concentrations. 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 like simplicity and high sensitivity of the sensor make it recommendable for medical diagnosis and clinical applications for the determination of cholesterol levels 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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References

[1].N.B. Myant, The Biology of Cholesterol and Related Steroids, Willium Heinemann, London, (1981).

[2].D.S. Fredrickson, R.I. Levy, J.B. Wyngarden, D.D. Fredrickson (Eds.), The Metabolic Basis of Inherited Disease, McGraw-Hill, New York, (1972).

[3].B Wang, J Huang, M Li, X Zhou, Multifunctional sensing film used for fiber optic cholesterol sensor, Proc. SPIE, Vol. 7278, (2009).

[4].Flink, T. N., Oraevsky, A., Jacques S. L, Autofluorescence detection of oxidized LDL in monocytes: a novel risk factor for the assessment of atherosclerosis, Proc. SPIE., Vol. 2679, 34, (1996).

[5].Yan, Y. H., Xu, Y. H., Li, S. P., Development of fiber optical biosensor of cholesterol based on fluorescence quenching, Chinese Journal of Biomedical Engineering, 23(1), 13, (2004).

[6]. Lee, I. N., Pinto D., Arriaga, E. A., Zhang, Z., Dovichi, N., Picomolar Analysis of Proteins Using Electrophoretically Mediated Microanalysis and Capillary Electrophoresis with Laser-Induced Fluorescence Detection, J. Anal. Chem., 70, 4546, (1998).

[7]. Cacheux, P. L., Menard, G., Quang, H. N., Weinmann, P., Jouan, M., Dao, N. Q., Quantitative Analysis of Cholesterol and Cholesterol Ester Mixtures Using Near-Infrared Fourier Transform Raman Spectroscopy, Appl. Spectrosc., 50, 1253, (1996).

[8].Wong, W.W., Hachey, D.L., Clark, L.L., Zhang, S., Llaurador, M., Piond, W.G., An improved HPLC method to purify erythrocyte cholesterol for estimation of invivo cholesterol synthesis using the deuterium method, Appl. Radial Isotopes, 45, 529, (1994).

[9].Agullo, E. and Susna, B., Gas-liquid chromatographic determination of total and free cholesterol in egg pastas, Food Res. Int., 29, 77, (1996).

[10].Lia G., Liao, J.M., Hu G.Q., Ma, N.Z., Study of carbon nanotube modified biosensor for monitoring total cholesterol in blood, Biosens. Bioelectron., 20, 2140, (2005).

[11].S. W. James, R. P. Tatam, Optical fiber long period grating sensors: characteristics and applications, Meas. Sci. Technol., Vol. 14, No. 5, 49, (2003).

[12].Jaw-Luen Tang, Jien-Neng Wang, Chemical Sensing Sensitivity of Long-Period Grating Sensor Enhanced by Colloidal Gold Nanoparticles, Sensors, 8, 171, (2008).

[13].Ignacio Del Villar, Ignacio R. Matías and Francisco J. Arregui, Philippe Lalanne, Optimization of sensitivity in Long Period Fiber Gratings with overlay deposition, Optics Express, Vol. 13, No. 1, (2005)

[14].Yinping Miao, Kaikiang Zhang, Yujie Yuam, Bo Liu, Hao Zhang, Yan Liu, and Jianquan Yao, Agarose gel-coated LPG based on two sensing mechanisms for relative humidity measurement, Applied Optics, Vol. 52, No. 1, (2013).

[15].Pesaramelli Karteek, Macharla Sravanthi, Anishetty Ranjith, Chitosan: A biocompatible polymer for pharmaceutical applications in various dosage forms, Intl. J Of Pharmacy & Technology, Vol. 2, Issue 2, 186, (2010).

[16].Young In Cho, Hong Kyoon No, and Samuel P. Meyers, Physicochemical Characteristics and Functional Properties of Various Commercial Chitin and Chitosan Products, J. Agric. Food Chem., Vol. 46, 3839, (1998).

[17].Wenshui Xia, Ping Liu, Jiali Zhang, Jie Chen, Biological activities of chitosan and chitooligosaccharides, Food Hydrocolloids, (2010).

[18].C. Bobby Mathews, T. M. Libish, J. Linesh, P. Biswas, S. Bandyopadhyay, K. Dasgupta, P. Radhakrishnan., A Biosensor for the Detection and Estimation of Cholesterol Levels based on Long Period Gratings,Sensors & Transducers, Vol. 149, Issue 2, 83, (2013).