No amount of experimentation can ever prove me right; a single experiment can prove me wrong.

-Albert Einstein

Chapter 5

Integrated Optical Waveguide Sensor with Lab-on-a-Chip Device Platform for Detecting Glucose Concentration in Blood Plasma
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5.1 Introduction
In the recent years, the assessment of accurate glucose level variation in fluid samples like blood plasma is a major challenge in the field of clinical diagnostics. We see that conventional way of measuring glucose level however, at present causes delays between sampling and analysis by analyzing samples at a clinical laboratory. Addressing to this matter, in this chapter we have developed a new miniaturized technique for detection of glucose concentration in blood plasma using Poiseuille's equation of viscous flow and light propagation through the optical waveguide. The technique is safe and capable of providing accurate result in terms of enhanced sensitivity. It is found that although tremendous investigation has been expanded in the development of wide range of accurate optical sensors in numerous applications including bio-sensing [1][2] and chemical detection [3], there is an immense need for an accurate sensor with fast detection which can provide patients with highly useful information of glucose concentration throughout the day. In spite, of the fact that researchers have demonstrated many techniques for glucose level detection using tools such as auto analyzer based on centrifuge system [4] and homecare glucose meter [5] but it is seen that the glucose meters vary in accuracy depending on multiple factors including patient’s technique in trying and finger cleanliness, and the chemistry and cross-reactivity with interfering substances. When selecting the optimal glucose meter, not only much aspects from the patient’s lifestyle and other health treatment be taken into account, but also the glucose meter systems must also be assessed in detail to ensure the minimum risk of interference [6]. Further, auto analyzer entail more time (order of few minutes) [4]. This chapter explores that the integration of an optical planar waveguide based sensor platform with LOC promises enhanced functionality and
performance such as high sensitivity, compactness and lower manufacturing expenses by the realization of such structures with silicon based material systems.

5.2 Sensing Concept and Design
In the proposed design of detecting glucose concentration, the blood samples were obtained from the eye-vein of rat and collected in the heparanized tube which acts as the reservoir and then it is pushed into the LOC using a syringe pump. Fig. 5.1 illustrates the proposed concept of rapid diabetes detection technique using optical waveguide sensor housed in a Plastic Cylindrical Enclosure (PCE). Using microfluidic LOC device the separated plasma from alloxan-induced diabetic rat blood is incorporated into the PCE through an interfacing tube between LOC and PCE. The LOC is installed within a fluidic network that includes interfacing capillary tube connected between LOC and optical waveguide sensor.

Fig-5.1: Block diagram showing the proposed concept of rapid diabetes detection using optical waveguide sensor.
In this technique, on-chip plasma separation is targeted to replace the classical bench top centrifugation. Fig. 5.2 shows the complete photograph of the experimental arrangement with LOC mounted on the specially developed optical set up along with micrometer adjustment for alignments.

5.2.1 Lab-on-a-Chip

The two main factors that take part in the choice of detection method for a lab-on-a-chip (LOC) application are sensitivity and scalability to smaller dimensions, especially with the increasing demands placed on sensors/detectors as volumes decrease. LOC is a commercially available microfluidic chip (Product code: 15-1503-0168-02, microfluidic ChipShop GmbH, Stockholmer Str.20D-07747 Jena, Germany, dated: 05-03-2013) of size 75.5 mm x 25.5 mm x 1.5 mm). It consists of chamber volume of 25 μl, a luer interface for blood loading, a support channel with a cross section of 300 μm x 100 μm for the transfer of the blood on top of a separation membrane [7]. An integrated

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plasma/serum generation chip for development of a sensor system is shown Fig 5.3 (a). Whereas, Fig. 5.3 (b) shows the close-up of one plasma/serum generation unit.

**Fig-5.3:** (a) Integrated plasma/serum generation chip for development of sensor system.

(Product code: 15-1503-0168-02, microfluidic ChipShop GmbH, StockholmerStr.20D-07747 Jena, Germany) and (b) Close-up of one plasma/serum generation unit

5.2.2 Design of sensor with interfacing capillary tube

**Fig-5.4:** Schematic of the planar waveguide sensor structure
For the design of the sensor, an optical planar waveguide sensor structure is considered as depicted in Fig. 5.4. As discussed earlier in chapter 3, the structure consists of a guiding (core) layer of Silicon Oxynitride (SiON) [Refractive Index (R.I), \( n_c = 1.46 \)]. This is deposited on silica-on-silicon substrate and sensing region of refractive index, \( n_s \), as a cladding and an outside medium (air) with refractive index \( n_a \) that is less than R.I of core. As observed earlier in chapter-3 and chapter-4 respectively, the change of the refractive index of the sensing layer results in the change in effective refractive index of the modes propagating in the planar waveguide sensor structure. Based on the Simple Effective Index Method (SEIM) [8], as discussed in detail in section chapter-2, and using the boundary conditions for Transverse Electric (TE) modes, the fractional power \( W(z) \) that remains inside the core along \( z \)-axis is obtained as [9],

\[
W(z) = W'(0)e^{-\frac{2\pi r}{\lambda}}
\]

(5.1)

where \( W'(0) = \left( \frac{0.0018}{n_s - 1.3315} \right) W(0), W(0) = \frac{k^2 \pi^2}{\Gamma^2} \), \( k = \frac{2\pi}{\lambda} \) and \( \Gamma \) is a function of waveguide parameters and \( \lambda = \) wavelength. The normalized power \( W(z)/W(0) \) versus length of the waveguide \( (z) \) is shown in Fig. 5.5. Although the design is being done for different cladding layer thickness \( (x_2-x_1) \sim 850 \mu m, 950 \mu m \) and \( 1050 \mu m \), it is seen that, \( W(z)/W(0) \) decreases with increase of \( z \) and becomes almost constant for \( z > 50,000 \mu m \). Further, we find that the variation of \( W(z)/W(0) \) for \( (x_2-x_1) = 950 \mu m \) is almost close to that for \( (x_2-x_1) = 1050 \mu m \). So we have chosen \( (x_2-x_1) = 950 \mu m \) and \( z = 50,000 \mu m \) respectively.

In this technique the accurate length of the optical waveguide sensor and designing of the interfacing tube is an essential part because we need to maintain the input pressure that generates inside the interfacing capillary tube for flowing of the blood plasma and filling up of the PCE which is illustrated in the next section. The blood plasma which gets collected in the PCE (via the interfacing tube) acts as the sensing layer (or, cladding layer) of the optical waveguide sensor for detection of plasma glucose level.
Fig-5.5: Normalized power versus length of the wave guide along $z$ direction for different $(x_2-x_1)$ ~ 850 $\mu$m, 950 $\mu$m and 1050 $\mu$m with $n_{\text{sub}}=1.45$, $n_s=1.329$ and $n_c=1.46$ respectively.

Fig. 5.6 shows the capillary interfacing tube of length $L_{\text{interface}}$ connected with LOC and PCE. The blood plasma is flowing through the tube under a constant pressure with the principle of Newton's law of viscous flow, following the Poiseuille's equation.
The input pressure $P_m$ required to inject the blood into LOC is determined as $P_m \sim 25000$ Pa, for maintaining the velocity ($v$) of blood plasma flowing at all points inside LOC to be $2 \times 10^{-6}$ liter/sec as specified by microfluidic ChipShop [7].

For designing the interfacing tube, at first we have taken the viscosity ($\sim 3.35 \times 10^{-3}$ Pa.s) of human blood, as the viscosity of blood plasma of human is almost constant with glucose level (90 mg/dl- 400 mg/dl). We have also measured viscosity of blood plasma of rat having glucose level varied from 90 mg/dl to 400 mg/dl by using a conventional viscometer (Viscometer labtech, LT 730) and plotted which confirms almost constant variation with glucose level as shown in Fig. 5.7. It is found that viscosity of human blood plasma is three times more than that of rat blood and therefore difficult to separate the plasma effectively from pure blood of rat [10]. We have mixed 80% distilled water with pure blood of rat to make the viscosity of blood of rat equivalent to that of human blood.

![Viscosity vs Glucose Level](image)

**Fig-5.7:** Viscosity versus glucose level for blood of rat and human blood as measured by a conventional viscometer.

For determining the pressure on LOC ($P_{Lab}$) and sensor input pressure ($P_{input sensor}$), we assume a cylindrical layer of liquid of radius $x$, flowing through a capillary tube of
radius \( r \), the velocity of flow at all points on this cylindrical layer is taken to be same. The schematic of the plasma flowing inside the interfacing capillary tube of length \( L_{\text{interface}} \) is shown in Fig. 5.8 (a). The velocity distribution profile is shown in Fig. 5.8 (b).

![Parabolic velocity profile](image)

(a)

![Velocity distribution curve](image)

(b)

**Fig-5.8:** (a) Schematic of the plasma fluid flow inside the interfacing capillary tube of length \( L_{\text{interface}} \) and (b) Velocity distribution curve.

If \( v \) is the velocity, as the velocity of the layers in contact with the walls of the tube is zero and goes on increasing towards the axis, it is obvious that the liquid inside the imaginary cylinder is moving faster than outside it and the backward tangential force due to the outer slower, moving liquid on the inner faster moving liquid is in accordance with the relation 1, given by

\[
\eta \frac{dv}{dx} \cdot L_{\text{interface}} = 0
\]

where \( \eta \) is the coefficient of viscosity of the liquid, surface area (A) of the cylindrical shell of radius \( x \) is equal to \( 2\pi x \cdot l \), \( L_{\text{interface}} \) is the length of the capillary tube interfacing with the sensor and \( \frac{dv}{dx} \) is the velocity gradient, as shown in Fig. 5.8 (b). We consider
the pressure difference at the two ends of the capillary tube be $P_{\text{input sensor}}$ and $P_{\text{lab}}$. Then the forward force on the cylindrical shell in the direction of flow is:

$$(P_{\text{input sensor}} - P_{\text{lab}}) \times \pi x^2$$

This tends to accelerate the motion of the liquid. Therefore, if the motion of the liquid is steady, following Poisseule Equation [11] we obtain:

$$\eta \cdot 2\pi L_{\text{interface}} \frac{dv}{dx} = -(P_{\text{input sensor}} - P_{\text{lab}}) \times \pi x^2$$

The negative sign indicates that the two forces are in opposite direction. On integrating for $v$, we obtain:

$$v = \frac{-(P_{\text{input sensor}} - P_{\text{lab}})x^2 + C_1}{4\eta L_{\text{interface}}}$$  \hspace{1cm} (5.3)

where $C_1$ is a constant of integration.

Now when $v=0$, $x=r$ because the layer in contact with the sides of the tube are stationary.

Therefore, $C_1 = \frac{(P_{\text{input sensor}} - P_{\text{lab}})r^2 + C_1}{4\eta L_{\text{interface}}}$ \hspace{1cm} (5.4)

Hence, $v = \frac{(P_{\text{input sensor}} - P_{\text{lab}})(r^2 - x^2)}{4\eta L_{\text{interface}}}$ \hspace{1cm} (5.5)

This is the velocity of flow of the liquid at a distance $x$ from the axis of the tube.

Volume of the interfacing capillary tube is $= \pi r_{\text{interface}}^2 L_{\text{interface}} v$ \hspace{1cm} (5.6)

where $r_{\text{interface}}$ is the radius of the capillary tube to be fitted with outlet of LOC. In order to determine the pressure on LOC ($P_{\text{lab}}$) and Sensor input pressure ($P_{\text{input sensor}}$), we assume another co-axial cylindrical shell of the liquid, of radius $(x + dx)$. The cross sectional area between the two shells is $2\pi x dx$. Since, $v$ is the velocity of flow of the liquid in between the two shells, the volume of the liquid flowing per second through the cross sectional area is $dv = 2\pi x dx v$. If we imagine the whole of the tube to be made of such like cylindrical shells, the volume $v$ of liquid flowing through the capillary tube in unit time is obtained by integrating Eq. (5.5) for $v$ between $x=0$ and $x=r$ and is obtained as:
\[ v = \int_{0}^{r} 2\pi x \cdot dx \cdot v = \pi \left( \frac{P_{\text{input sensor}} - P_{\text{lab}}}{8\eta L_{\text{interface}}} \right)^{4} \] (5.7)

Or, \( \left( P_{\text{input sensor}} - P_{\text{lab}} \right) = \frac{8\eta L_{\text{interface}}}{\eta^{4}} \) (5.8)

Therefore, the input pressure of the sensor generated by the interfacing capillary tube with perpendicular radii of curvature \( r \) is calculated with the Poiseuille's equation and is obtained as:

\[ P_{\text{input sensor}} = P_{\text{lab}} + \frac{8\eta L_{\text{interface}}}{\eta^{4}} \] (5.9)

Now following Hagen-Poiseuille law \([11]\) which states, the flow rate is proportional to the pressure difference \( \Delta P = P_{\text{input sensor}} - P_{\text{lab}} \) between the ends of the capillary tube and the fourth power of its chip radius, \( r_{\text{chip}} \) we find,

\[ P_{\text{lab}} = P_{\text{in}} - \frac{8\eta L_{\text{chip}}}{\eta^{4}} \] (5.10)

But \( P_{\text{in}} \) is the input pressure required to inject the fluid into the LOC \(-250 \text{ mbar} \) as specified by Microfluidic chipShopp \([7]\), \( \eta \) is the coefficient of viscosity, \( L_{\text{interface}} \) is the length of the interfacing capillary tube, \( L_{\text{chip}} \), length of channel carrying blood plasma in chip, \( r_{\text{chip}} \) is the radius of chip. The velocity \( (V) \) of plasma flow in microfluidic chip is \( 2 \times 10^{-9} \text{ m}^{3}/\text{sec} \).

Now, volume of channel carrying blood plasma in chip is \( = \pi r_{\text{chip}}^{2} L_{\text{chip}} \cdot V \) (5.11)

For evaluating \( L_{\text{interface}} \), it follows from Eq. (5.6) and Eq. (5.11), that the length of the interfacing capillary tube can be expressed as:

\[ L_{\text{interface}} = \frac{r_{\text{chip}}^{2}}{r_{\text{interface}}^{2}} L_{\text{chip}} \] (5.12)

For \( r_{\text{chip}} = 0.26 \text{ mm}, r_{\text{interface}} = 0.075 \text{ mm} \) and \( L_{\text{chip}} = 9 \text{ mm} \), from Eq. (5.12), the length of the capillary tube that interfaces with LOC is obtained as, \( L_{\text{interface}} = 115 \text{ mm} \) to maintain the flow rate as same as that in the channel of LOC. Further, substituting in Eq. (5.10) for \( \eta = 0.00035 \text{ Pa.s; } V = 2 \times 10^{-9} \text{ m}^{3}/\text{sec} \), the pressure on the Lab-on-a-chip is obtained as, \( P_{\text{lab}} = 24999.916 \text{ Pa} \). From Eq. (5.9), substituting for \( r_{\text{interface}} = 0.075 \text{ mm} \) to be fitted with outlet of LOC. We get the input pressure that is maintained inside the capillary tube to
cause the blood plasma flow into PCE is derived using Poiselle's equation and obtained as:

\[ P_{\text{input sensor}} = P_{\text{Lab}} + \frac{8 \eta L_{\text{interface}}}{r_{\text{interface}}^4} \approx 25001.71362 \text{ Pa} \quad (5.13) \]

For evaluating the length of the PCE, it follows from Eq. (5.6) that the volume (\( v \)) of liquid flowing through the capillary tube in unit time is obtained by integrating Eq. (5.7) for \( v \) and obtained as:

\[ v = \frac{\pi \left( P_{\text{input sensor}} - P_{\text{lab}} \right)}{8 \eta L_{\text{interface}}} \quad (5.14) \]

Now if \( r_{\text{sensor}} \) is the radius of PCE (that holds the waveguide sensor inside) and \( P_{\text{atm}} \) is the atmospheric pressure we get:

\[ \frac{\pi \left( P_{\text{input sensor}} - P_{\text{lab}} \right)}{8 \eta L_{\text{interface}}} = \frac{\pi \left( P_{\text{input sensor}} + P_{\text{atm}} \right)}{8 \eta L_{\text{interface}}} \quad (5.15) \]

Here, \( r_{\text{sensor}} \) the radius of PCE is designed for \(-1000 \times 10^{-6} \text{ m}\) by considering waveguide propagation characteristics which is discussed later. Atmospheric pressure \( (P_{\text{atm}}) \) is negligible, as a result from the above expression (5.15), the length of the plastic cylindrical enclosure (PCE) is obtained as,

\[ l_2 = \frac{L_{\text{interface}} \left( P_{\text{input sensor}} + P_{\text{atm}} \right) r_{\text{interface}}^4}{\left( P_{\text{input sensor}} - P_{\text{lab}} \right) r_{\text{sensor}}^4} \approx 50000 \mu\text{m} \quad (5.16) \]

5.2.3 Sensitivity

As we see that for sensor performance, sensitivity is an important factor for determining the actual accuracy of the sensing system. As described in detail in chapter 3, the condition for maximum sensitivity to changes in the cover/sensing(cladding) layer also known as the waveguide sensitivity \( S_w \) (i.e. the rate of change of effective refractive index \( N \) with respect to refractive index \( n_s \) of the sensing medium) is expressed as,

\[ S_w = \frac{\delta (\beta/k_0)}{\delta n_s} \quad (5.17) \]

where, \( \beta/k_0 \) = effective refractive index and \( n_s \) is the sensing region refractive index.
Fig. 5.9 shows the waveguide sensitivity versus core refractive index \((n_c)\) with \(n_s=1.45\) and \(x_2-x_1=950 \mu m\) for proposed waveguide structure (solid line) and previous works [18] (dotted line). It is found that the waveguide sensitivity of the proposed structure is almost independent with core RI. The high waveguide sensitivity of the \(~4.1\) compared to the earlier reported is \(~40\) times more than that of the previous works as reported by previous authors [18].

![Graph showing waveguide sensitivity versus core refractive index](image)

**Fig- 5.9:** Waveguide sensitivity versus \(n_c\) with \(n_s=1.45\) and \(x_2-x_1=950 \mu m\) for proposed waveguide structure (solid line) and previous works [18] (dotted line).

### 5.2.4 Limit of Detection (LOD)

Sensitivity is related to the limit of detection (LOD), which is defined as the minimum amount of concentration or mass of the biochemical substance that can be detected by the sensor over the background signal. Limit of detection depends on the resolution of the sensor. In the proposed technique of glucose level detection, we see that the measured values of the normalized power \((W(z)/W(0))\) versus glucose level for three group of rats \(A_1, A_2\) and \(A_3\) (having almost same body weight) as discussed in section 5.5.1 are in good agreement with the theoretical result. It is seen that the signal at the output of the sensor is independent of RI of the SR for \(n_s \geq n_c\), as the signal will no
longer be confined in the core region. So, the limit of detection (LOD) of our sensor will be restricted up to core RI and for our experimental setup as shown in Fig. 5.3, Limit of Detection (LOD) is 1.333-1.46 which is found to be more than the earlier reported works [12].

5.2.5 Estimation of detection time

The detection time ($\tau_D$) is an important parameter for detecting the concentration of glucose in blood plasma. In the proposed technique $\tau_D$ comprises of separation time, $t_{sep}$, propagation time, $t_p$, refilling time, $t_r$, and the time required by the detector to give the response. As such, $\tau_D$ can be written as:

$$\tau_D = \text{Separation time } (t_{sep}) + \text{Propagation time } (t_p) + \text{Refilling time } (t_r) + \text{Detector response } (t_{response})$$

(5.18)

where, $t_{sep} = 20 \text{ sec}[7]$ and

$$t_p = \frac{\text{channel length}}{\text{velocity of plasma in channel}}$$

(5.19)

Also we find that the rate of flow of plasma in channel depends upon the radius of the chip ($r_{chip}$) and the velocity ($V$) of plasma flow. Therefore, we may put this as:

**Velocity of plasma in channel is**

$$V = \frac{\pi r_{chip}^2}{\pi r_{chip}^2}$$

$$V = 2 \times 10^{-9} \text{ m}^3/\text{sec}$$

$$= \frac{(0.26)^2 \times 3.14 \times 10^{-6}}{2 \times 10^{-9}}$$

$$= 9.421 \text{ mm/sec}$$

(5.20)

After substitution of (5.20) in (5.19), $t_p$ is found to be $\sim 0.95$ seconds.

Thus,

**Refilling time, $t_r$**

$$t_r = \frac{\pi r_p^2 PCE}{V}$$

$$= \frac{3.14 \times (250 \times 10^{-6})^2 \times 5 \times 10^{-2}}{2 \times 10^{-9}}$$

$$= 4.2 \text{ sec}$$
And \( t_{\text{response}} \) is very much negligible \( \sim 800 \) ps \([12]\).

Therefore, the total time required by the sensor for its detection is found to be \( \sim 25.85 \) seconds.

### 5.2.6 Estimation of sample volume

The sample volume of the sensor was set at 0.141 ml. Its volume is calculated using the relation,

\[
\text{Sample volume} = \text{Inside volume of PCE} - \text{Volume of waveguide sensor}
\]

where volume of waveguide sensor = volume of waveguide core and volume for wafer and lower cladding on silicon substrate. Hence,

\[
\text{Sample volume} = \pi R^2 L_{\text{PCE}} - W^2 L_{\text{PCE}} - W_{\text{substrate width}} L_{\text{substrate}} T_{\text{substrate}} \tag{5.21}
\]

where \( T_{\text{substrate}} = 310 \mu m [300 \mu m (\text{Si}) + 10 \mu m (\text{SiO}_2)] \)

\( L_{\text{substrate}} = L_{\text{PCE}} = 50,000 \mu m \)

\( W_{\text{substrate width}} = 1000 \mu m \)

\( r_{\text{PCE}} = 1000 \mu m \)

\( W = \text{core area} = 50 \mu m \) (since \( x_1 = 25 \mu m \), therefore \( 2x_1 = 50 \mu m \))

Substituting these values in Eq. (5.21), we get

\[
\text{Sample volume} = 141.375 \times 10^{-9} \times 10^3 \text{ liter} \\
= 0.000141375 \text{ liter} \\
= 0.141 \text{ ml}
\]

In the proposed sensor, we find that the simplicity of its construction and the high precision of this planar waveguide optical glucose sensor make it an alternative to previously reported commercially available glucose sensors. Especially the sample volume of 0.141 ml and the 25.85 sec measurement time are the highest specifications for its sensing purpose compared to the currently available glucose sensors. Table 5.1 shows the design parameters for integrating the waveguide sensor with LOC.
Table 5.1: Design parameters used for the proposed planar waveguide optical sensor

<table>
<thead>
<tr>
<th>Design parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>(L_{\text{sensor}})</td>
<td>(50000 \mu m)</td>
</tr>
<tr>
<td>Core width ((2x_1))</td>
<td>(50 \mu m)</td>
</tr>
<tr>
<td>Cladding width ((x_2-x_1))</td>
<td>(950 \mu m)</td>
</tr>
<tr>
<td>Substrate refractive index ((n_{\text{sub}}))</td>
<td>(1.45)</td>
</tr>
<tr>
<td>Sensing region refractive index ((n_{\text{s}}))</td>
<td>(1.329)</td>
</tr>
<tr>
<td>Refractive index of core ((n_{\text{c}}))</td>
<td>(1.46)</td>
</tr>
<tr>
<td>Viscosity ((\eta))</td>
<td>(0.00035 \text{ Pa.s})</td>
</tr>
<tr>
<td>(P_{\text{in}})</td>
<td>(250 \text{ mbar})</td>
</tr>
<tr>
<td>(V)</td>
<td>(2\times10^{-9} \text{ m}^3/\text{sec})</td>
</tr>
<tr>
<td>(r_{\text{chip}})</td>
<td>(260 \mu m)</td>
</tr>
<tr>
<td>(r_{\text{interface}})</td>
<td>(75 \mu m)</td>
</tr>
<tr>
<td>(L_{\text{chip}})</td>
<td>(9000 \mu m)</td>
</tr>
<tr>
<td>(P_{\text{Lab}})</td>
<td>(24999.916 \text{ Pa})</td>
</tr>
<tr>
<td>(P_{\text{imp sensor}})</td>
<td>(25001.71362 \text{ Pa})</td>
</tr>
<tr>
<td>(r_{\text{sensor}})</td>
<td>(1000 \mu m)</td>
</tr>
</tbody>
</table>

5.3 Fabrication of waveguide sensor

Although different technologies are being employed for producing integrated optic waveguide-based sensors which depend on the application but application of SiON has been mainly motivated by its excellent optical properties, such as low absorption losses in the visible and near infrared wavelength range as mentioned in chapter-2. The sensor planar waveguide of length 50,000 \(\mu m\) was fabricated using SiON as the waveguide core material. Fig. 5.10 shows the SEM image of the waveguide sensor. The fabrication process is described in detail in chapter 3. The embedded SiON waveguide core of width 50 \(\mu m\) has been deposited by plasma enhanced chemical vapor deposition and the RI has been tailored by controlling different gas concentrations (SiH\(_4\), N\(_2\)O, and NH\(_3\)).
Patterns are transferred by standard photolithography and have been developed and etched by RIE using CF₄ and O₂.

Fig-5.10: SEM image of the waveguide sensor

5.4 Experiment for the diabetic study

5.4.1 Preparation of the rat model
Adult both male and female Wistar rats 5-6 months, weighing 200-250 gm were housed in groups of six with ad libitum access to food and water. Animals were maintained in a temperature-controlled room with 12 hour alternating light and dark cycles. Experiments were performed during the light period of the cycle and were conducted in accordance with the "Principles of Laboratory Animal care" (National Institute of Health, USA publication 85-23, revised 1985) and were approved by institutional animal ethical committee.

5.4.1.1 Induction of Experimental Diabetes
Total 36 rats were fasted for 72 hours before drug administration. The next morning animals were anesthetized with Phenobarbital sodium at a dose level of 40 mg/kg body weight before injecting alloxan monohydrate. A single intraperitoneal injection of alloxan in fixed dose in freshly prepared 10x10⁻³ mol/l sodium citrate [13], (pH 4.5) was delivered through the eye to induce diabetes.

5.4.1.2 Fixation of doses for induction of diabetes
Different timing and doses of Alloxan administration were made to develop an efficacious drug dosage. Fig. 5.11(a) and Fig. 5.11(b) show the chemical structure and IUPAC nomenclature of Alloxan. Specifically, different doses (60 mg/kg, 90 mg/kg, and 110 mg/kg) had different effects on diabetes. In the proposed experiment for diabetic study, rats were routinely treated daily (Monday through Friday).
5.4.2 Assessment of Diabetes and Hyperglycemia
Rats were considered diabetic and included in the study if they had plasma glucose levels >170 mg/dl [14]. After confirmation of the development of diabetes by measuring the fasting plasma glucose concentration, the animals were returned to their home cages. In addition, the fasting plasma glucose concentration was determined after 72 hours to confirm that hyperglycemia was maintained during this period. The plasma glucose level was estimated for every 3rd day, 5th day, 8th and 11th day. The plasma glucose estimation was also done orally in the pathological laboratory (Tezpur University Health Centre). The development of diabetes was verified with results obtained by the state of the art device used in the Tezpur University Centre, the Siemens Dimension ® clinical chemistry system.

5.5 Measurement Setup
After the fabrication of the designed planar waveguide sensor, as discussed above and verification of diabetes the optical power measurement is executed for detection of glucose level using the developed sensor device in the proposed experimental setup as shown in Fig. 5.3 above. A stabilized Helium Neon laser beam of wavelength 0.6328 μm and power 1 mW was launched to the optical fiber (which is butt-coupled to waveguide sensor inside PCE) by aligning with the focusing lens for light input into the

Fig-5.11: (a) Chemical structure of Alloxan and (b) IUPAC nomenclature.
11 days. In case of $A_2$ rats with dose 90 mg/kg body weight the measured power varies from zero day to 11 days as glucose level increase with number of days as shown in Table-5.2. In case of $A_3$ rats with dose 110 mg/kg body weight, the glucose level increases from 108 mg/dl to 823 mg/dl (which is severe diabetic) within 3 days and because of that the measured power is reduced to 40 % with respect to input power.

**Fig-5.13:** Refractive index ($n_s$) of blood plasma versus glucose level (as measured by Abbe Refractometer) for all three groups of rats $A_1$, $A_2$ and $A_3$

**Table 5.2:** Effect of Alloxan on glucose level of rats for different days

<table>
<thead>
<tr>
<th>Number of days</th>
<th>$A_1$ rat with alloxan dose 60 mg/kg body weight glucose level</th>
<th>$A_2$ rat with alloxan dose 90 mg/kg body weight glucose level</th>
<th>$A_3$ rat with alloxan dose 110 mg/kg body weight glucose level</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>98</td>
<td>105</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>176</td>
<td>823</td>
</tr>
<tr>
<td>5</td>
<td>108</td>
<td>295</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>117</td>
<td>385</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>115</td>
<td>420</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 5.3: Detection performance comparison for different blood glucose concentration measurement system.

<table>
<thead>
<tr>
<th>S.L. No.</th>
<th>Authors/ Year</th>
<th>Technique/ process used</th>
<th>Detection time</th>
<th>Sample volume</th>
<th>Sensitivity</th>
<th>Limit of Detection (LOD)</th>
<th>Accuracy</th>
<th>Capability of measuring glucose samples concentration(C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Lin, J. F. et al./(2009) [18]</td>
<td>Electro-optically modulated circular polariscope</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>as low as 0.2 gm dl-1</td>
</tr>
<tr>
<td>2.</td>
<td>Suhandy, D. et al./(2012) [19]</td>
<td>ATR-THz spectroscopy</td>
<td>-</td>
<td>300 µL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>Cubuk, S. et al. (2013) [20]</td>
<td>Boronic acid based fluorescence sensor</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>over a concentration of 0.1 ppm–0.7 ppm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>Yasin, M. et al. (2010) [21]</td>
<td>Intensity modulated fiber optic</td>
<td>-</td>
<td>50 ml</td>
<td>0.0103mV/(%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Pockevicius, V. et al. (2013) [22]</td>
<td>Using Inter-digital Electrodes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Error does not exceed 10 %</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Tura, A. et al. (2010) [23]</td>
<td>Electromagnetic sensor</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>~0.22 mV/(mg/dL) (~78–5,000 mg/dL)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Lin, Y. H. et al. (2013) [24]</td>
<td>a solid-state sensor, micropump, and micro-valve integrated into a microfluidic device</td>
<td>200 µl</td>
<td>49.16 mV pH^1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Worsley, G.J. et al. (2008) [25]</td>
<td>Phenylboronic-based sensor</td>
<td>40 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9.</td>
<td>V, A., Kumar, N. (2013) [26]</td>
<td>Wavelet Transform and Neural Networks</td>
<td>20 µl</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10.</td>
<td>Our proposed technique</td>
<td>Based on integrated waveguide sensing technology and integrated with LOC</td>
<td>25.85 secs</td>
<td>0.141 ml</td>
<td>4.1</td>
<td>0-850 mg/dl</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Interdigital Electrodes [19], phenylboronic-based sensor [22], Wavelet Transform and Neural Networks [23] but obtaining accurate measurements is a key concern. Very recently, in ref [24] we find that although the author has developed a sensor for blood glucose estimation using inter digital electrodes which eliminate finger pricking and any possible risk of infection but the sensor lacks from accuracy compared to our proposed sensor structure. Table 5.3 shows the detection performance comparison for different blood glucose concentration measurement system. From the reported work in ref [21], we see that for measurement of glucose concentration the author has made use of microfluidic chip integrated with an electrolyte-insulator-semiconductor sensor. But it is important to note that in this existing technique the amount of sample consumption is more, around ~200 μl compared to our sample volume ~0.141 ml for its sensing application.

5.7 Conclusion
In this chapter, the study and design of a rapid diabetic detection technique using integrated optical waveguide sensor with Lab-on-a-chip device platform was carried out using Poiseuille’s of viscous flow and light propagation through optical waveguide. The technique developed was implemented for detection of glucose level in alloxan-induced rat blood with administration of different doses of Alloxan. It is found that the intra peritoneal injection of alloxan dose 90 mg/kg body weight induces the diabetes starting from normal glucose level (110 mg/dl) in zero days to 420 mg/dl in 11 days. The integrated approach developed requires very minimal sample volume of ~0.141 ml which is less than the work found in the recent reported literature [24]. The concept presented in this work promises low cost online diagnostic technique for detection of glucose level in the near future.
5.8 List of References


