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POROUS BASED IMMUNOSENSOR FOR DETECTION OF LDL MOLECULES FROM BLOOD SERUM USING ARRAY OF CANTILEVER BEAM

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ABSTRACT

In this work, the NiO thin film based array of Cantilever structure for the detection of low-density lipoprotein (LDL) is proposed. To detect the LDL molecules in the applied blood serum, the highly selective Anti-apolipoprotein B (AAB-100) act as the phosphate buffer solution (PBS) and it is coated at the tip of the porous NiO thin film based array of cantilever beam. The diffusion length and deflection of cantilever beam are analyzed for the different loads. Presence of five LDL molecules will make the displacement of 3.089e-4μm. While the amount of LDL molecule is increased, the displacement also gets increased.

Keywords: Anti-apolipoprotein-B, Array of cantilever, Immunosensor, Low density lipoprotein (LDL), Nickel oxide, Porous.

1. INTRODUCTION

1.1. LDL Particle

In blood, cholesterol is carried by different types of lipoproteins. Among them, Low density lipoprotein (LDL) has high risk as it is related with heart diseases and other ailments like atherosclerosis[2,3]. LDL molecule consists of cholesterylesters, underesterified cholesterol and a single protein molecule and apolipoprotein B-100. Large number of LDL molecules can lead to artery blockage which leads to cholesterol accumulation in the artery walls. In order to diagnose, quantity of LDL should be monitored frequently. The normal range and density of LDL molecule is 130mg/dl and 1.04g/ml. Several methods are used to measure the quantity of LDL molecules [1]. One of the important methods is Fried Wald equation method which is also known as indirect method. In this method, LDL molecule is calculated by subtracting the HDL and triglycerides from total cholesterol. But it does not provide an accurate result[4].

Some of the direct methods are Nuclear Magnetic Resonance method and Ultra centrifugation method. Direct methods are high cost and slow. The best alternate method is biosensor based detection method which provides low cost with good sensitivity and selectivity. When the biological element is used as an antigen or antibody fragment on the biosensor, then the sensor is called immuno sensor. The immuno sensor is based on the interaction of LDL molecule with its specific antibody [4].

The size of the LDL molecule is in the range of 22nm to 27nm which is very small and it cannot be separated by using the biofilter[5]. With the help of the antigen and antibody interactions, the amount of LDL particles can be detected.

1.2 Antigen and Antibody Interaction

Different methods are available to detect the Antigen and antibody interaction. One of the important indirect techniques is ELISA technique. In this technique, the antigen is immobilized to adsorb the antibody (LDL) molecules. ELISA technique requires the following three parameters (1) plastic bead or micro titer wall, (2) antigen which must be immobilized on the plastic bead or micro titer wall, and (3) antibodies which we want to predict. The process is shown in Figure 1. In step 1, antigen is immobilized. In Step 2, sample is applied to the well.

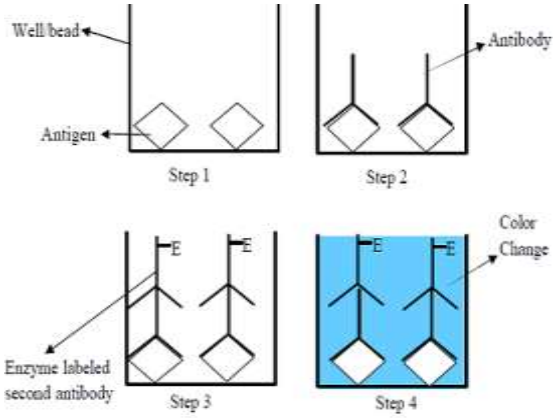


Figure 1: Schematic of antigen and antibody interaction

In step 3, if the sample contains antibody which are specific to the antigen, then the antibody will attach automatically to that antigen. In step 4, chromic substance is added to bead or wall and color changes are obtained. Finally, obtained color change depends only on the amount of antibodies present in the applied sample[9].

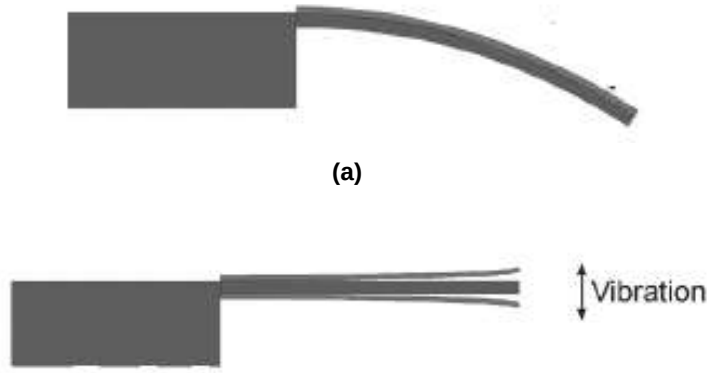
1.3 Micro-Cantilever Sensor

In a recent years, Cantilever based sensors provide very attractive environment to detect the biomolecules present in the fluid environme

nt. The immunoglobin molecule can be detected using array of cantilever beam[7].Micro cantilever sensors with selective coatings for target immobilization are ideal for the application of bio sensor. The deflection of the cantilever with respect to mass can be accurately determined by running the instrument in the following two modes.

(i)Static mode - In this mode the deflection/bending of the cantilever will occur due to surface charge stress and it is caused by analyte interaction.

(ii) Dynamic mode - In this mode, the resonance frequency of the cantilever will get change with respect to the mass load. During adsorption, the mass of the cantilever will shift the resonance frequency to lower frequencies.



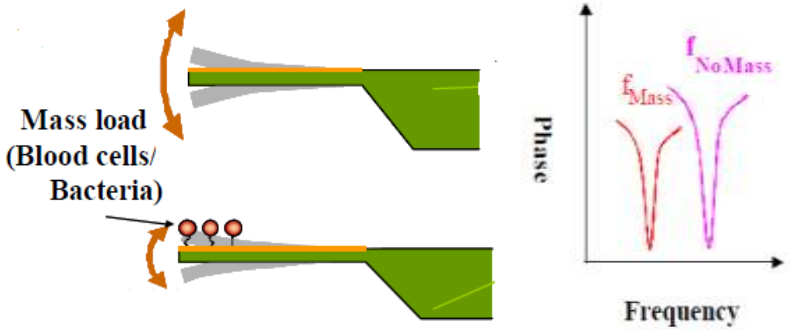
(a)

(b)

Figure 2: Operational modes of Cantilever beam

Figure 2a represents the static mode operation, figure 2b represents the dynamic mode operation of the cantilever beam[8].The adsorption of molecule on the surface of micro-cantilever can be studied by observing a shift in the resonance frequency and monitoring a cantilever bending. Array of cantilever structure can be used to improve the accuracy of the LDL detection. In this paper, static mode of cantilever beam can be used.

In figure 3a represents the deflection of the cantilever beam with and without mass. Without mass, the cantilever makes the displacement in larger extent. When the blood is introduced to the cantilever beam, LDL gets adsorbed and the mass of the beam is increased. With mass, the deflection of the beam is decreased when compared with cantilever without mass. In figure (3-b), the curve on the right hand side indicates the phase value with respect to frequency when no mass is added to the cantilever beam [7].



(a) **(b)**

Figure 3: Resonance frequency detection

The curve on the left hand side indicates the phase value when mass is added to the cantilever beam. The phase of the cantilever will be decreased when mass is added to the sensor. Because the vibration of the cantilever is not strong when no mass is added to the surface.

1.4 Deflection Detection Method

The cantilever can be operated in static or dynamic mode. In static mode, detection of analyte absorption can be analyzed with the help of cantilever bending. In dynamic mode, their analyte adsorption can be analyzed with the help of change in resonance frequency. Several methods are there to detect the deflection beam. Some of the important methods are optical lever, interferometry, capacitive sensor, and piezoelectric/piezoresistive cantilever [9].

1.5 Matrix Immobilization

The effectiveness of bio detection is highly depends on nature and quality of matrix can be utilized to bioreceptor for immobilization. Different types of immobilization are there to perform the matrix array for immobilizing the bio receptor on the surface of the array of cantilever.One of the important application of array of cantileveris to detect the cancer cells, in order to detect the cancer cell peptides can be placed on the surface of the array of the cantilever, here the Peptides can be coated on the surface and it is immobilized by using the self-assembled monolayers (SAMs). The coated peptides will detect the specific ligands which is related to the cancer. The detection can be done on the principle of operation static mode. The operation of cantilever means that deflection or bending of the cantilever beam can be made as a result of analyte-ligand interaction of the tip of the cantilever beam. This analyte-ligand interaction will change the surface stress of the array of cantilever beam, so the designed cantilever beam starts to bend[9].

2. DESIGN CONCEPT

In order to detect the LDL particles from the filtered molecules, the porous silicon with PBS coated array of cantilever is designed and simulated. The AAB is coated at the tip of the array of the cantilever beam. The AAB is used as a PBS solution in which the antibody is immobilized using EDC/NHS linkage. The LDL molecules which are present on the applied blood sample can be adsorbed only at the tip of the NiO thin film based cantilever beam where the PBS is immobilized. Using Chemical engineering module the diffusion concentration of the cantilever beam is simulated and also the diffusion length is analyzed. Using solid mechanics, the array of PBS coated NiO thin flim based cantilever beam is designed and simulated to analyze the displacement of the array of cantilever beam with respect to the given force. The PBS solution is highly sensitive to adsorb only the LDL particles. Then the free end deflection of array of cantilever beam is a direct measure of the antigen and antibody interaction. So that the obtained displacement is only depends on the amount of LDL particles were present on the applied blood serum.

If the blood sample is applied on the cantilever beam, the LDL molecules are adsorbed at the tip of the cantilever beam. As a result the cantilever will make a displacement. Due to the presence of array like structure this device will require the less blood sample and produce the effective result.

The array of micro cantilever based immuno sensor is designed by coating the PBS solution on the porous Nio thin film. All of the cantilever beam surface will be coated by PBS solution. If the blood serum is injected into the surface which is covalently immobilized antibody, then antigen-antibody interaction will take place. Based on this interaction the amount of LDL adsorption will be increased on the surface of the cantilever beam. Obviously the deflection/bending of the cantilever beam will goes to a large extent. The bio detection can be done only based on the effect of immobilization.

Material used: NiO material is used to design cantilever beam and top surface of the array of cantilever beam is coated with PBS solution. The Poisson ratio and the young's modulus of the Nio thin film is 0.384 and 171.8Gpa respectively.[6]

Physics used: The physics behind the cantilever beam is solid mechanics. Boundary load is applied to analyse the displacement with respect to mass of the antigen and antibody interaction.

Size used: The length, width and height of the cantilever beam is 1.3μm, 0.02μm and 0.05μm.

2.1 PBS coated Nio thin film

The surface concentration on the PBS coated Porous thin film can be designed using species transport in porous media from the chemical engineering module.Figure 4 shows that the simulation result of surface concentration for PBS coated porous based Nio thin flim. The surface concentration is increased with respect to time.

Diffusion

The transfer of particles from higher concentration into Lower concentration can be determined by using the following formula.

$$X= \text{sqrt}(2Dt)$$

Where, X is the difffusion length,

t is the diffusion time

D is the diffusion constant.

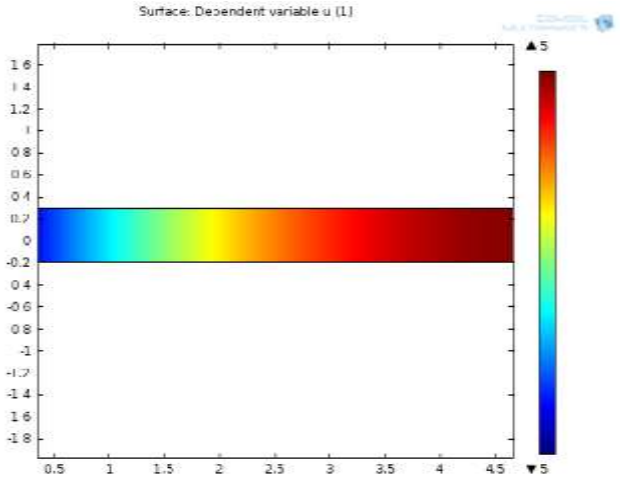


Figure 4: surface concentration on PBS coated Nio thin film

The value of the diffusion length and surface concentration of the antigen and antibody interaction is increased with respect to time. The Table-I indicates the value of the diffusion length for the different time duration.

Table 1. Diffusion length of LDL molecules

S.No	Time duration (sec)	Diffusion length (μm)
1	1	8.94
2	2	12.64
3	3	15.49
4	4	17.88
5	5	20

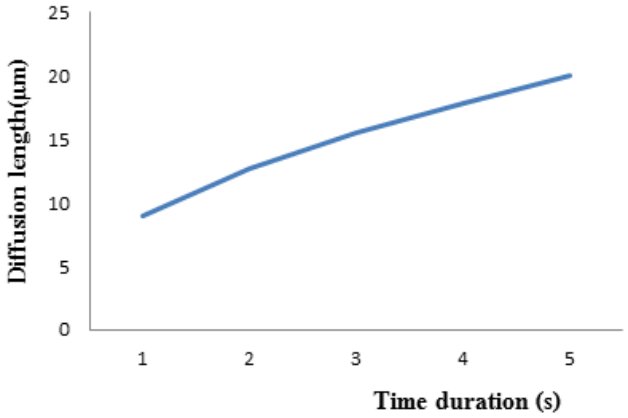


Figure 5: Diffusion length Versus time period.

Figure 5 shows that if the time duration is increased, it will adsorb more antibodies on its surface. With the help of increased surface concentration the amount of LDL molecules in the applied blood serum is detected. At the corner of the surface the concentration is more to adsorb the LDL molecule.

2.2 Micro cantilever sensor:

The cantilever structure can be designed and simulated by using the solid mechanics from the structural mechanics module, and then analyze the displacement with respect to the antigen-antibody interaction.

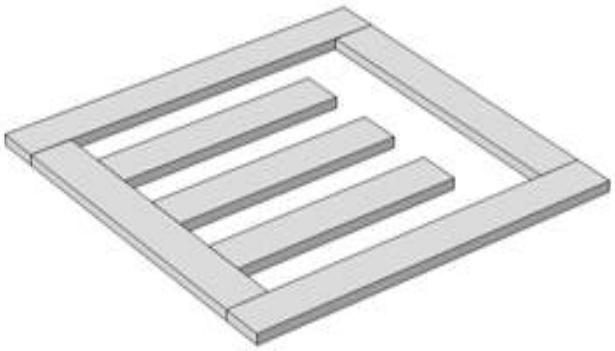


Figure 6: 2D model of the Array of cantilever beam.

In figure 6, the 2D view represents that the two dimensional view of the cantilever beam. Using the Bezier polygon the above structure can be designed.

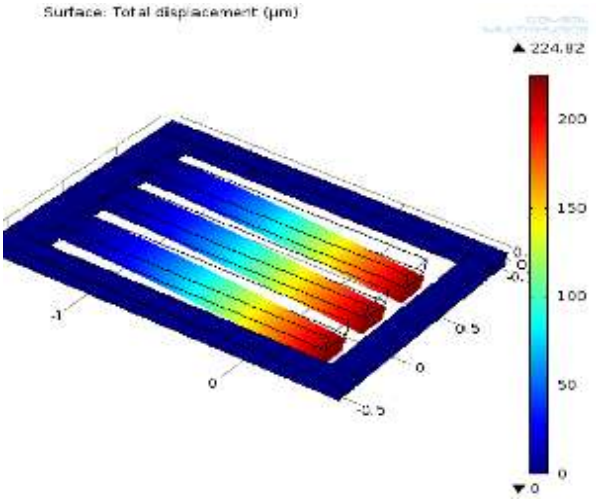


Figure 7: Displacement of array of cantilever sensor.

In Figure 7, it will shows that the deflection or displacement can be made only on the antigen and antibody interaction. Boundary load can be applied in terms of force.The equivalent force of the LDL molecules can be calculated using the formula,

$$F= ma$$

Where, F is the force per unit area,(N/m).

m is the mass of the load (mg/dl).

a is the acceleration due to gravity(9.8 m/s^2).

Figure 8 shows that the cross sectional view of the array of cantilever beam after the respective boundary load is applied at the tip of the cantilever beam.

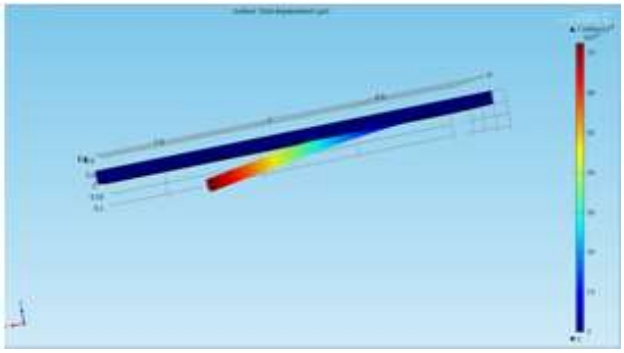


Figure 8: Cross sectional view of the displacement of the Array of cantilever beam.

3 MATHEMATICAL MODELLING

3.1 Boundary Load Calculation

In order to calculate the boundary load in terms of force the molecular weight of the LDL molecule is 3 million Dalton can be used. To convert the Dalton into force, first the Dalton can be converted into mass (g or Kg) and then the obtained mass will convert into force(N/m).

The conversion factor of mass into force is 0.000009806N. Using Dalton to mass conversion, the obtained molecular weight of one LDL molecule is 4.9816 e-18 gms or 4.9816 e-15 mg. Conversion for different number of molecules is shown in table 2.

Table 2. Conversion of Dalton to molecular weight

S.No	No of molecules	Molecular weight (mg)
1	5	24.98e-15
2	10	49.816e-15
3	15	74.724 e-15
4	20	99.632 e-15
5	25	124.54 e-15

The conversion of mass into force can be done using the factor 0.000009806 N. For example the force of the 5 LDL molecules is 2.44e-19 N/m.

3.2 Displacement Calculation

The displacement of the cantilever beam can be calculated by using the beam formula. Based on the position of the load to be applied on the surface of the cantilever the formula will be varied. That means the load may be applied on either, the tip or the entire top surface or the half of the top surface. In order to obtain the maximum deflection for small amount of sample, the load can be applied only at the tip of the cantilever beam. Because the small amount of LDL molecules can produce the large displacement.

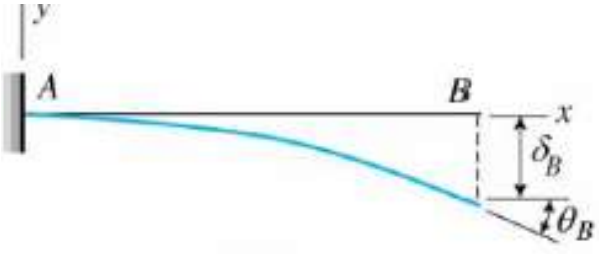


Figure 9: Displacement of cantilever beam

Figure 9 shows that position of the load, the cantilever can be loaded only at the tip of the surface. The formula for calculating the maximum displacement of cantilever beam at the tip is given by,

$$\delta max = \frac{Pl^3}{3EI}$$

Where, P is the applied load,

l is the length of the cantilever beam,

E is the Young's Modulus and

I is the moment of inertia.

The young's modulus can be varied depends on the materials used. For Nio thin film, the young's modulus can obtained as 171.8 Gpa. The moment of inertia of our designed cantilever beam is 100.83~101.Moment of inertia can be calculated using the formula,

$$I = \frac{bh^3}{12}$$

Where, b is the width of the cantilever beam and

h is the height of the cantilever beam.

Using the above equations, the obtained moment of inertia of cantilever beam is 100.83~101.

Table 3. Comparison of theoretical and practical displacement

S.No	No of Molecules	Force (N/m)	Theoretical displacement (μm)	Displacement (μm)
1	5	$2.44e^{-019}$	3.089e-4	3.6228e-5
2	10	$4.88\ e^{-019}$	6.1788e-4	7.2456e-5
3	15	$7.327\ e^{-019}$	9.277e-4	1.0879e-4
4	20	$9.769\ e^{-019}$	7.15e-3	1.4505e-4
5	25	$1.2212\ e^{-018}$	3.51e-2	1.8132e-4

The above mentioned table represents the value of the obtained results using formula and simulation.

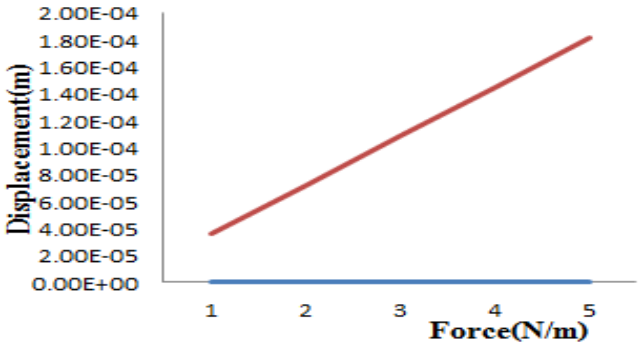


Figure 10: Relationship between the displacement and the number of particles

Figure 10 shows that if the number of particles were increased, then the displacement also increased. Due to their small size the cantilever beam will be more flexible to detect the LDL molecules in the applied blood.

Figure 11 shows that the comparison between the theoretical and practical displacement. The obtained results, shows that some variations with the theoretical value. But the obtained value will also be increased when the force is

increased. By using this highly selective cantilever the deflection can be made only on the adsorption of LDL molecules. Due to this parallel arrangement the reliability of the cantilever will be increased.

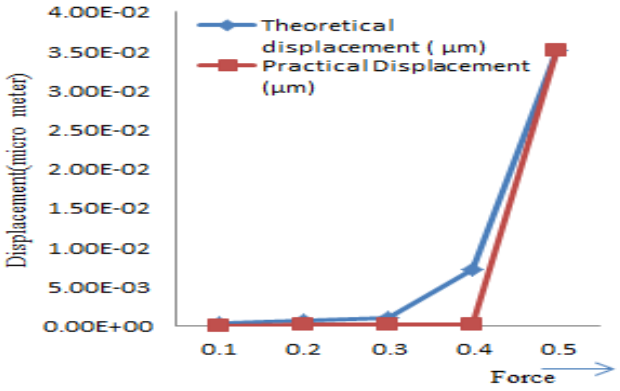


Figure 11: Comparison of theoretical displacement and practical displacement

4. CONCLUSION

In this paper the diffusion of LDL particles on the surface of the cantilever beam can be analyzed using the chemical engineering module. Then the array of micro cantilever based bio sensor was designed using COMSOL multiphysics, Force can be applied as the boundary load. The deflection of the cantilever beam can be analyzed for different boundary load. Finally the Theoretical and practical displacement was compared and graph was plotted.

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