



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI  
PhD-17 SHORT ABSTRACT OF THESIS

Name of the Student : Malaya Mili

Roll Number : 196106014

Programme of Study : Ph.D.

Thesis Title: Development of aptasensors for detection of Shiga like toxin 1 (Stx1) and Shiga like toxin 2 (Stx2) from *Escherichia coli*.

Name of Thesis Supervisor(s) : Prof. Pranab Goswami

Thesis Submitted to the Academic Division : BSBE

Date of completion of Thesis Viva-Voce Exam : 12-06-2026

Key words for description of Thesis Work : Aptamer, Shiga toxin, Biosensor, interdigitated microelectrode.

---

**SHORT ABSTRACT**

Shiga toxin-producing *Escherichia coli* (STEC) are among the leading causes of foodborne illnesses worldwide, contributing to significant morbidity and mortality. The Shiga-like toxin 1 (Stx1) and Shiga-like toxin 2 (Stx2) expressed by STEC are the main virulence factors responsible for bloody diarrhoea, haemolytic uremic syndrome (HUS), and haemorrhagic colitis (HC). The severity of the diseases necessitates rapid, sensitive, portable, and low-cost diagnostic tools to strengthen on-field food safety monitoring, improve disease prognosis and guide timely intervention. Current detection methods typically involve culture-based identification, PCR-based molecular diagnostics and antibody-based immunoassays. These methods involve complex procedures, long assay times, and require advanced instrumentation as well as trained personnel. Moreover, antibody production is costly, prone to batch-to-batch variability, and antibodies often lose activity under fluctuations in temperature, pH, or storage conditions. These limitations hinder their applications in point-of-care settings, where the on-field conditions vary, and the standard storage conditions are not always feasible to maintain. Aptamers, owing to their high selectivity, strong thermal stability, low production costs, and ease of functionalization, are being increasingly recognized as ideal biorecognition elements for biosensing applications. In this thesis work, aptamer-based impedimetric biosensors for the detection of Stx1 and Stx2 were developed using interdigitated microelectrodes and their sensing performances were evaluated using electrochemical impedance spectroscopy (EIS). The aptasensor for Stx1 was fabricated using a highly selective aptamer developed via standard Systematic Evolution of Ligands by Exponential Enrichment (SELEX) method. The aptamer was characterized, and then immobilized on a chain-type interdigitated microelectrode to develop the aptasensor. The microelectrode was fabricated following advance techniques such as electron beam deposition, chemical wet etching and

photolithography. The developed aptasensor exhibited a limit of detection (LOD) of 2.88 pM, a linear dynamic range of 10 - 450 pM and a sensitivity of 107.02  $\Omega$ /pM. To validate the practical application of the aptasensor it was tested in real sample using cow milk. The sensor showed the recovery in the range of 97.5% to 103.5%. In the next phase of the work an aptamer against Stx-2 was developed. In this part of the work, the aptamer was however, identified from a computationally designed aptamer library following a systematic in-silico approach comprising of generation of aptamer library, prediction of secondary and tertiary structures of the aptamers, molecular docking, and molecular dynamic simulation. The selected aptamer was validated using circular dichroism (CD) and isothermal titration calorimetry (ITC). The *in silico* designed aptamer for Stx2 was then used as the biorecognition element for the development of the Stx2 biosensor using a wave-type interdigitated microelectrode. The wave type microelectrode fabrication followed the same techniques described for the chain type electrode. The developed sensor exhibited an LOD of 4.63 pM, a linear range of 10 – 400 pM and a sensitivity of 97.03  $\Omega$ /pM. To validate the practical application of the aptasensor it was tested in real sample using cow milk. The sensor showed recovery in the range of 93.06% to 108.4%.

