



IoT-Based Biosensing Kit for Salivary Protein Estimation

Moushami D¹, Rachna V², B Srinidhi³, Surekha Bhangari⁴, Ganga Holi⁵

Student, Department of Computer Science and Engineering (ICB), K. S. Institute of Technology, Bengaluru, Karnataka, India¹

Student, Department of Computer Science and Engineering (ICB), K. S. Institute of Technology, Bengaluru, Karnataka, India²

Student, Department of Computer Science and Engineering (ICB), K. S. Institute of Technology, Bengaluru, Karnataka, India³

Assistant Professor, Department of Computer Science and Engineering (ICB), K. S. Institute of Technology, Bengaluru, Karnataka, India⁴

Professor, Department of Computer Science and Engineering, Alliance University, Bengaluru, Karnataka, India⁵

Abstract: The rapid advancement of Internet of Things (IoT) technology has enabled the development of intelligent healthcare systems capable of real-time monitoring and analysis. This paper presents the design and implementation of an IoT-based biosensing kit for estimating protein concentration in human saliva using a colorimetric detection technique. The proposed system integrates an ESP32 microcontroller, TCS3200 colour sensor, Biuret reagent, I2C LCD display, and the ThingSpeak cloud platform to provide a portable, cost-effective, and non-invasive protein detection solution. The colorimetric reaction converts biochemical changes into measurable optical signals, which are processed using a calibration model to estimate protein concentration and transmitted to the cloud for real-time visualization and analysis. The developed prototype was evaluated through a preliminary experimental study by comparing salivary protein concentration before and after chanting, demonstrating the feasibility of the proposed approach for behavioural and physiological monitoring. Experimental results demonstrated successful protein estimation, reliable cloud-based data monitoring, and ease of operation, indicating the potential of the proposed system for point-of-care diagnostics and future smart healthcare applications.

Keywords: Internet of Things (IoT), ESP32, Salivary Protein Detection, Biosensor, TCS3200 Colour Sensor, Biuret Assay, ThingSpeak, Non-invasive Diagnostics.

I. INTRODUCTION

The increasing demand for affordable, portable, and non-invasive healthcare solutions has accelerated the integration of Internet of Things (IoT) technologies with biomedical sensing systems [5], [9]. Conventional laboratory-based diagnostic methods often require sophisticated equipment, trained professionals, and significant processing time, making them less suitable for continuous health monitoring and point-of-care applications. Consequently, there is a growing need for smart healthcare devices that can provide rapid, accurate, and real-time diagnostic information while remaining cost-effective and easy to operate.

Proteins present in biological fluids serve as important biomarkers for diagnosing various physiological and pathological conditions [4]. Changes in protein concentration may indicate kidney disorders, infections, inflammatory diseases, malnutrition, and other medical conditions. Among the available biological samples, saliva has gained considerable attention because it offers a painless, non-invasive and convenient alternative to blood-based testing [9]. Salivary diagnostics eliminate the need for trained personnel during sample collection and reduce patient discomfort while enabling frequent health monitoring.

Recent advancements in biosensor technology and embedded systems have significantly enhanced the capabilities of portable healthcare devices [3], [5]. The integration of Internet of Things technology with biosensing systems enables automatic data acquisition, wireless communication, cloud storage, and remote monitoring. Such systems improve



diagnostic efficiency by providing real-time access to patient information and allowing healthcare professionals to monitor physiological parameters without requiring repeated laboratory visits.

The proposed research focuses on developing an IoT-enabled biosensing kit for detecting protein concentration in human saliva using the Biuret colorimetric assay. During the biochemical reaction, proteins react with copper ions in an alkaline medium to produce a violet-coloured complex [1],[2]. The intensity of this colour is directly proportional to the protein concentration and is measured using a TCS3200 colour sensor. The acquired data are processed by an ESP32 microcontroller, displayed locally on an I2C LCD module, and simultaneously transmitted to the ThingSpeak cloud platform for real-time visualization and storage.

In addition to protein detection, the developed system investigates variations in salivary protein concentration before and after chanting activities. Chanting is widely recognised as a mindfulness practice that contributes to stress reduction, emotional stability, and overall well-being. However, quantitative analysis of its physiological effects remains limited. The proposed IoT-enabled biosensing kit provides an effective platform for measuring these variations through continuous monitoring of salivary protein levels.

The proposed system emphasises portability, affordability, user-friendliness, and cloud connectivity. Unlike conventional laboratory equipment, the developed prototype is compact and suitable for healthcare centres, educational institutions, rural clinics, and home-based monitoring applications. The integration of IoT technology further enhances remote healthcare services by enabling real-time data visualization and historical record management through cloud-based storage.

The primary objectives of this research are as follows:

- To develop a portable IoT-enabled biosensing kit for detecting protein concentration in human saliva.
- To analyse variations in salivary protein concentration before and after chanting activities.
- To implement a colorimetric detection mechanism using the Biuret assay and TCS3200 colour sensor.
- To provide real-time monitoring and cloud-based visualization using the ESP32 microcontroller and ThingSpeak platform.
- To develop a cost-effective, portable, and user-friendly healthcare monitoring system suitable for point-of-care diagnostic applications.

The main contribution of this work is the development of a portable IoT-enabled biosensing kit that combines the Biuret colorimetric assay, TCS3200 colour sensor, ESP32 microcontroller, and ThingSpeak cloud platform for real-time salivary protein estimation. Unlike conventional laboratory-based protein estimation methods, the proposed system provides a compact, low-cost, and non-invasive solution suitable for point-of-care healthcare applications.

II. SYSTEM DESIGN AND METHODOLOGY

A. System Architecture

The proposed IoT-based protein detection system is designed to provide real-time monitoring of salivary protein concentration through an integrated biosensing platform. The complete system consists of four major modules: the sensing unit, processing unit, display unit, and cloud communication module. These modules work together to acquire biochemical data, process sensor readings, and provide real-time visualization both locally and remotely.

Initially, the saliva sample is mixed with the Biuret reagent to produce a colour change proportional to the protein concentration present in the sample. The TCS3200 colour sensor detects the intensity of the developed colour and converts it into corresponding frequency values. These sensor readings are processed by the ESP32 microcontroller, where calibration equations are applied to determine the protein concentration. The calculated results are displayed on an I2C LCD module and simultaneously transmitted to the ThingSpeak cloud platform through Wi-Fi connectivity for remote monitoring and historical data analysis.

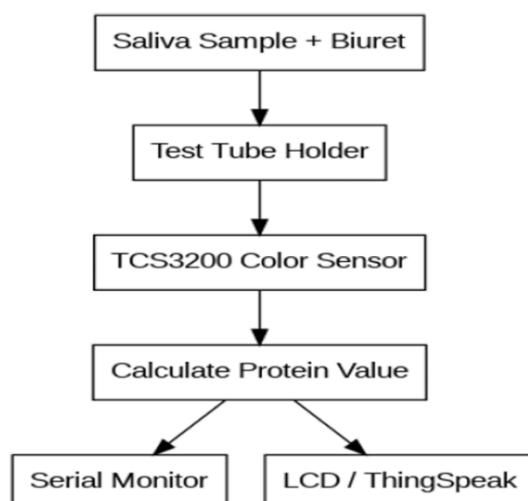


Fig. 1. System Architecture of the Proposed IoT-Based Protein Detection Kit

B. Hardware Components

The hardware implementation of the proposed system comprises several low-cost electronic components that collectively perform biochemical sensing, signal processing, and wireless communication. The ESP32 microcontroller serves as the central processing unit because of its high processing capability, integrated Wi-Fi module, and low power consumption [6]. The TCS3200 colour sensor is employed to detect the colour intensity produced during the Biuret reaction [7]. An I2C LCD module provides local visualization of the calculated protein concentration, while ThingSpeak serves as the cloud platform for storing and monitoring the collected data.

TABLE 1: HARDWARE COMPONENTS USED IN THE PROPOSED SYSTEM

Component	Specification	Purpose
ESP32	Wi-Fi Enabled Microcontroller	Data Processing and Cloud Communication
TCS3200	RGB Colour Sensor	Detection of Colour Intensity
Biuret Reagent	Chemical Reagent	Protein Detection
I2C LCD Display	16 × 2 LCD	Local Display of Results
ThingSpeak	IoT Cloud Platform	Remote Monitoring and Data Storage
Power Supply	5V DC	System Power Source

C. Colorimetric Detection Principle

The proposed biosensing system employs the Biuret colorimetric assay for determining protein concentration in saliva samples. During the biochemical reaction, peptide bonds present in proteins react with copper ions in an alkaline medium, producing a violet-coloured complex. According to Beer-Lambert's law, the intensity of the developed colour is directly proportional to the protein concentration.

The TCS3200 colour sensor measures the reflected light intensity corresponding to the violet colour developed during the reaction. The obtained RGB frequency values are then processed using a calibration equation developed through linear regression analysis. This approach enables accurate estimation of protein concentration while maintaining low system complexity and cost.

D. Software Methodology

The software for the proposed system was developed using the Arduino IDE. The ESP32 microcontroller continuously acquires colour sensor readings and performs pre-processing to eliminate environmental noise. The processed RGB



values are converted into protein concentration using a predefined calibration equation obtained during experimental analysis.

The calculated values are displayed on the LCD module for immediate user feedback. Simultaneously, the ESP32 establishes a wireless connection with the ThingSpeak cloud platform and uploads the measured protein concentration for remote monitoring. Cloud integration enables continuous data logging and graphical visualization, allowing users to access historical measurements from any location with internet connectivity[8].

E. Functional Modules

The complete system operation is divided into five functional modules:

1. **Data Acquisition:** The TCS3200 colour sensor captures the RGB frequency values corresponding to the colour produced during the Biuret reaction.
2. **Data Pre-processing:** Multiple sensor readings are collected and averaged to minimise environmental disturbances and improve measurement accuracy.
3. **Protein Concentration Estimation:** The calibrated regression model converts RGB frequency values into protein concentration expressed in mg/mL.
4. **Local Visualization:** The calculated protein concentration is displayed on the I2C LCD module for instant observation.
5. **Cloud Communication:** The ESP32 uploads the measured data to the ThingSpeak cloud platform through Wi-Fi, enabling real-time visualization and long-term storage.

F. System Workflow

The operational workflow of the proposed system consists of the following sequential steps:

1. Collect saliva sample.
2. Mix the sample with Biuret reagent.
3. Allow colour development.
4. Detect colour intensity using the TCS3200 sensor.
5. Process sensor data using the ESP32 microcontroller.
6. Calculate protein concentration using the calibration model.
7. Display results on the LCD.
8. Upload data to the ThingSpeak cloud.
9. Monitor results through the cloud dashboard.



Fig. 2. Flowchart of the Proposed Protein Detection System

III. IMPLEMENTATION AND RESULTS

A. Hardware Implementation

The hardware implementation of the proposed IoT-based protein detection kit was carried out by integrating the ESP32 microcontroller, TCS3200 colour sensor, I2C LCD display, and the required biochemical reagents into a compact prototype. The ESP32 acts as the central controller responsible for sensor interfacing, data processing, wireless communication, and cloud connectivity.

The TCS3200 colour sensor was enclosed inside a custom-designed dark chamber to minimise the effect of ambient light during colour measurement. Saliva samples mixed with the Biuret reagent were placed inside the sensing chamber, where the colour sensor detected the intensity of the violet colour produced during the biochemical reaction. The sensor output was continuously monitored by the ESP32, which calculated the protein concentration and displayed the results on the LCD module. Simultaneously, the processed data were uploaded to the ThingSpeak cloud platform using the built-in Wi-Fi capability of the ESP32.

B. Software Implementation

The software was developed using the Arduino Integrated Development Environment (Arduino IDE). The ESP32 firmware continuously acquires RGB frequency values from the TCS3200 sensor and performs necessary preprocessing to reduce measurement noise. Multiple sensor readings are averaged to improve accuracy before calculating the protein concentration using a predefined calibration equation.

The developed program also establishes communication with the ThingSpeak cloud platform through Wi-Fi. Each measured value is periodically transmitted to the cloud, allowing users to monitor protein concentration remotely through



graphical visualization. The software architecture consists of sensor initialization, data acquisition, colour processing, protein estimation, LCD display, and cloud communication modules.

C. Calibration and Protein Estimation

The accuracy of the proposed system was evaluated through calibration using standard protein solutions of known concentrations. The relationship between colour intensity and protein concentration was analysed using linear regression. The resulting calibration model exhibited a strong linear relationship, enabling accurate conversion of sensor readings into protein concentration values.

The calibration curve demonstrated an approximate regression equation:

$$y = -0.0004x + 1.1081$$

with a coefficient of determination

$$R^2 = 0.987$$

indicating excellent agreement between the measured values and standard protein concentrations. The high coefficient of determination confirms that the proposed colorimetric sensing approach provides reliable protein estimation within the experimental range.

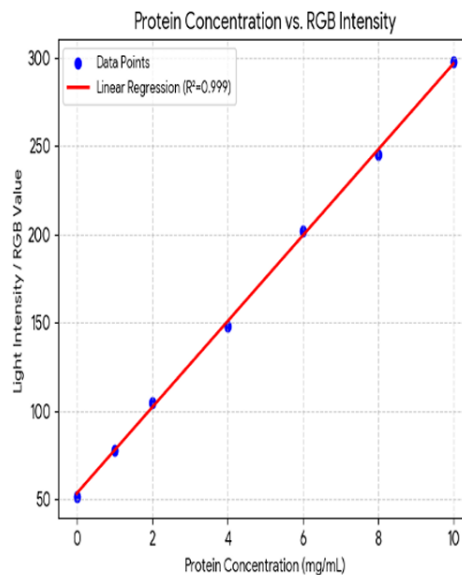


Fig. 3. Calibration Curve for Protein Concentration Estimation

D. Experimental Analysis

To evaluate the practical performance of the developed system, saliva samples were collected before and after a chanting session. Equal quantities of saliva were mixed with the Biuret reagent, and the developed colour intensity was measured using the TCS3200 sensor.

The obtained sensor readings were converted into protein concentration using the calibration equation. The processed results were displayed on the LCD module and uploaded to the ThingSpeak cloud platform for real-time monitoring.

The experimental observations indicated measurable variations in salivary protein concentration after chanting. These variations demonstrate the capability of the developed system to monitor short-term physiological changes through non-invasive biochemical analysis.



TABLE 2: EXPERIMENTAL OBSERVATIONS

Parameter	Observation
Sample Type	Human Saliva
Detection Method	Biuret Colorimetric Assay
Sensor Used	TCS3200 Colour Sensor
Controller	ESP32
Cloud Platform	ThingSpeak
Output	Protein Concentration (mg/mL)
Display	I2C LCD

E. Cloud Monitoring

One of the major advantages of the proposed system is its ability to provide real-time remote monitoring through Internet of Things technology. After protein estimation, the ESP32 uploads the calculated concentration values to the ThingSpeak cloud platform using HTTP communication over Wi-Fi.

The cloud dashboard provides graphical visualization of protein concentration, enabling users to analyse trends over time without manual data recording. Historical records can also be accessed for future analysis, making the proposed system suitable for continuous healthcare monitoring applications.

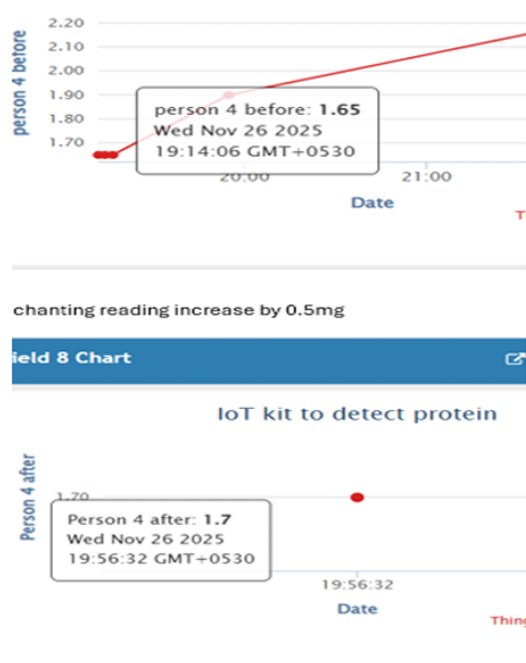


Fig. 4. ThingSpeak Dashboard Showing Real-Time Protein Concentration

F. Performance Discussion

The developed prototype demonstrates several advantages over conventional laboratory-based protein detection methods. The proposed system is portable, cost-effective, non-invasive, and capable of providing real-time monitoring through IoT connectivity. The integration of the ESP32 microcontroller with the TCS3200 colour sensor enables accurate protein estimation while maintaining low hardware complexity.

The calibration results indicate high measurement accuracy with a regression coefficient of $R^2 = 0.987$, demonstrating the reliability of the proposed colourimetric sensing approach. Furthermore, the cloud-based monitoring system allows users to access protein concentration data remotely, supporting telemedicine and point-of-care diagnostic applications.



TABLE 3: COMPARISON OF PROPOSED SYSTEM WITH CONVENTIONAL METHODS

Feature	Conventional Laboratory Method	Proposed IoT Kit
Sample Collection	Laboratory Based	Non-invasive Saliva Sample
Equipment	Expensive	Low Cost
Portability	No	Yes
Real-Time Monitoring	Limited	Available
Cloud Connectivity	Not Available	ThingSpeak Cloud
Remote Monitoring	No	Yes
Ease of Operation	Requires Skilled Personnel	User Friendly

IV. LIMITATIONS

Although the proposed IoT-based protein detection kit demonstrated promising performance, several limitations remain. The prototype was evaluated using a limited number of saliva samples, and broader clinical validation is required to establish its reliability. Environmental lighting conditions and variations in sample preparation may influence colour measurement accuracy. Additionally, the current implementation estimates total protein concentration only and requires an active Wi-Fi connection for cloud-based monitoring.

V. CONCLUSION

This paper presented the design and implementation of an Internet of Things (IoT)-enabled biosensing kit for detecting protein concentration in human saliva using a colorimetric detection technique. The proposed system successfully integrates an ESP32 microcontroller, a TCS3200 colour sensor, a Biuret assay, an I2C LCD display, and the ThingSpeak cloud platform to provide a portable, cost-effective, and non-invasive healthcare monitoring solution. The developed prototype accurately converts biochemical colour changes into measurable protein concentration values and enables real-time visualization through cloud connectivity.

The experimental evaluation demonstrated that the proposed system can effectively monitor variations in salivary protein concentration before and after chanting, highlighting its potential application in physiological and behavioural studies. The calibration results showed high measurement accuracy, validating the reliability of the proposed sensing approach. Furthermore, the integration of IoT technology enables continuous remote monitoring and historical data storage, making the system suitable for point-of-care diagnostics, telemedicine, and smart healthcare applications.

Future work will focus on improving the sensitivity of the biosensing module, integrating multiple biomarker detection capabilities, developing a dedicated mobile application for remote access, and incorporating machine learning algorithms to enhance predictive healthcare analysis and personalized health monitoring.

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**REFERENCES**

- [1]. M. J. Gorenstein and R. F. Bondy, "The Biuret Method for Protein Determination," *Analytical Biochemistry*, vol. 180, no. 2, pp. 200–205, 1989.
- [2]. S. S. Deshpande and S. Damodaran, "Spectrophotometric Quantitation of Proteins," *Food Proteins and Their Applications*, pp. 100–115, 1997.
- [3]. S. Lyu et al., "Optical Fiber Biosensors for Protein Detection: A Review," *Sensors*, vol. 22, no. 3, p. 1163, 2022.
- [4]. G. Riccardi et al., "Salivary Biomarkers in Oral Squamous Cell Carcinoma: A Proteomic Overview," *Cancers*, vol. 14, no. 6, p. 1462, 2022.
- [5]. T. Dong et al., "Advances in Electrochemical Biosensors Based on Nanomaterials for Protein Biomarker Detection," *Advanced Science*, vol. 9, no. 2, p. 2105074, 2022.
- [6]. Espressif Systems, "ESP32 Series Datasheet," Version 4.1, 2024.
- [7]. AMS AG, "TCS3200 Programmable Color Light-to-Frequency Converter Datasheet," 2023.
- [8]. MathWorks, "ThingSpeak Documentation," MathWorks Inc. [Online]. Available: <https://www.mathworks.com/help/thingspeak/>.
- [9]. T. Nonaka et al., "Saliva Diagnostics," *Clinical and Experimental Dental Research*, vol. 8, no. 3, pp. 364-372, 2022.
- [10]. Y. Kil, "Engineering and Computational Tools for Salivary Biomedicine," Ph.D. Thesis, California Institute of Technology, 2025.