

Development of a device to detect *in vitro* derived glycated proteins based on the fluorescence quenching properties of graphene oxide

N. A. Malagala¹, B. P. N. Kumara¹, W. P. P. Kaweesha¹, H. Y. R. Atapattu¹,
W. K. S. M. Abeysekera², N. Abeyasinghe³, J. K. D. S. Jayanetti¹

¹*Department of Instrumentation and Automation Technology, Faculty of Technology,
University of Colombo, Sri Lanka*

²*Department of Agricultural Technology, Faculty of Technology, University of Colombo, Sri Lanka*

³*Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka*

Glycated proteins (GPs), produced through the non-enzymatic glycation of proteins by glucose, are emerging biomarkers for early diabetes diagnosis. This study reports a fluorescence-based mechanism to detect *in vitro* derived GPs and its development into a prototype sensing device. Graphene Oxide (GO) was synthesized using the Improved Hummers method, while GPs were produced through a BSA-glucose model. Fluorescence studies were carried out in 96-well microplates ($n = 3$) using a 300 μL reaction volume containing 100 μL of GO (1.0 mg mL^{-1}), varying GP concentrations ($7.5 - 37.5 \mu\text{M mL}^{-1}$), phosphate buffer (pH 7.4), and distilled water (emission: 370 nm, excitation: 440 nm). By adopting the fluorescence quenching behaviour of GO, a GPs detection mechanism was established, and a prototype device was designed and developed using an Arduino Nano, UV LED (370 nm), collimator lens, narrow band pass filter (440 nm), and TSL257 light-to-voltage sensor. The structural components of the device were designed in SolidWorks and 3D printed to have a precise device configuration. The real-time results were displayed using an LCD (16×2). The fluorescence spectra revealed the quenching effect of GO against GPs, which is highly dose-dependent in the concentration range of $7.5 - 37.5 \mu\text{M mL}^{-1}$. The highest $|\Delta \text{Fluorescence Intensity}|$ was observed at 420 - 430 nm, with the secondary peak at 440 nm. The $|\Delta \text{Fluorescence Intensity}|$ exhibited a linear relationship with GP concentration ($R^2 = 0.9927$), validating GO as a potential sensing material. Further, the developed device (30 replicates per concentration) confirmed consistent light-to-voltage responses with minimum intervention against distilled water, buffer, and BSA while showing a baseline voltage of 0.03 V for GO, confirming its minor inherent fluorescence properties. The device demonstrated a strong linearity and precision (STD: 0.022 to 0.037 V) while exhibiting an average device accuracy of 94.88%. Further improvements in the repeatability and accuracy of voltage responses may lead to potential testing in biological matrices, allowing fabrication of a point-of-care device for early detection of diabetes.

Keywords: *Glycated proteins, Fluorescence quenching, Graphene oxide, Biosensor, Diabetes diagnosis*