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Ankan Dutta Chowdhury, Enoch Y. Park

Real-time monitoring of Sphingomyelinase (SMEnzyme) which converts sphingomyelin into ceramide, modulating membrane properties in cell and signal transduction, is a crucial biomarker for Niemann–Pick and several other diseases like atherosclerosis, multiple sclerosis, and HIV. In this study, we have presented an electrochemical method to detect SMEnzyme concentration in a faster and more sensitive way compared to other currently available commercial assays due to the successful amplification process of liposomal matrix. For the amplification process, a methylene blue (MB)-encapsulated sphingomyelin (SM)-based liposome has been constructed which can release of the encapsulated MB in presence of the target enzyme and can be detected on a working electrode. A gold-polyaniline embedded (Au-PAni) graphene quantum dots (N,S-GQDs) coated nanocomposites, prepared via interfacial polymerization and then self-assembly approach has been used to capture the released MB on a glassy carbon electrode for electrochemical Differential Pulse Voltammetric (DPV) analysis. To get the optimum capture through π–π stacking interaction of the released MB on the Au-PAni/N,S-GQDs nanocomposites, which has been used as the working electrode. Minimal cross-reactivity with similar phospholipase and proteins confirms the stable and non-leaky MB-liposome platform with low background signal and high specificity toward the SMEnzyme with a detection limit of 7.2 μU mL^{-1}. Taken together, the simplicity and high sensitivity of this present method offer huge potential in point-of-care diagnostics of SMEnzyme detection.