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Real-time monitoring of Sphingomyelinase (SMEEnzyme) 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 SMEEnzyme 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 SMEEnzyme with a detection limit of $7.2 \mu\text{U mL}^{-1}$. Taken together, the simplicity and high sensitivity of this present method offer huge potential in point-of-care diagnostics of SMEEnzyme detection.