

## **Non-Invasive Alzheimer's Diagnosis by Innovative BODIPY-Based Ocular Delivery**

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Alzheimer's disease (AD) is the leading cause of dementia worldwide, and early, non-invasive diagnostic strategies remain a major challenge. The retina represents a promising window for monitoring neurodegenerative alterations due to its anatomical and embryological similarities with the brain. BT1, a BODIPY-based fluorescent probe with high affinity toward tau aggregates, shows promising potential for AD detection; however, its hydrophobicity and poor aqueous solubility limit its biological applicability. Therefore, oil-in-water nanoemulsions (NEs) were developed as nanocarriers to improve BT1 solubilization, stability, and ocular delivery. NEs were formulated using almond oil and Tween 80, and optimized through ternary phase diagrams. The formulations were characterized in terms of size, polydispersity index (PDI), and  $\zeta$ -potential by Dynamic Light Scattering (DLS). Stability was evaluated at 4°C and 25°C and in biologically relevant media, including Dulbecco's Modified Eagle Medium (DMEM) and simulated lacrimal fluid. The internal organization of the NEs and BT1 interactions were investigated by fluorescence anisotropy using DPH. Preliminary cytotoxicity assays were also performed to assess biocompatibility. The optimized NEs showed a transparent and homogeneous appearance, droplet sizes below 100 nm, and narrow size distributions. The formulations maintained their physicochemical properties during storage, demonstrating good stability. Fluorescence anisotropy suggested interactions between BT1 and the hydrophobic domain of the NEs, potentially contributing to probe stabilization. Initial formulations showed limited cellular compatibility due to the high Tween 80 content; reducing surfactant concentration significantly improved biocompatibility while preserving the desired physicochemical properties. Overall, these results highlight the potential of NEs as stable and biocompatible carriers for BT1 ocular delivery, supporting their future application in non-invasive retinal imaging for Alzheimer's disease detection.