Abstract Book

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2.20. Protocol Design for the Method Using 2'7'dichlorodihydrofluorescin (DCFH) as the Fluorescent Probe to Detect the Nanoparticle Reactivity

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Understanding the oxidative stress generated from reactive particles is one key mechanism to clarifying the toxicity of these particles. However, there is a lack of a uniform and reliable method. In this study, we assessed the performances of different approaches and improved the existing methods to detect the NPs reactivity. We evaluated several commonly used chemicals to prepare the solution. We did cross-comparison of various concentrations of fluorescent probe and catalyst. We also compared different sonication medias to disperse NPs. Moreover, the proper sample suspension range was explored and ultra filtration was used to study the possible optical interference of using high sample concentration. Scan absorbance spectra over time were made to support our conclusions. A 2'7-dichlorodihydrofluorescin (DCFH) working solution with 0.5 unit/ml horseradish peroxidase (HRP) yielded repeatable results. Also, ethanol was chosen to prepare DCFH stock solution. Sodium buffer was preferred as the solvent for DCFH-HRP working solution. Based on our results, sonication in DCFH-HRP working solution would provide stable data with a relatively clean background than using a buffer or Tween 80 as the sonication solution. Moreover, to evaluate the reactivity of a certain type of NP, a relatively wide samples concentration range should be applied. Particle concentration in the magnitude of mg/ml was considered too high which may cause some interference. The protocol created could be used as a standard method in both academic and industry research because it is a feasible method that seems to yields reliable and repeatable results.