Optimization of the Synthesis of the Fluorescent Dye "Nile Red"

Surdokaite, Meda (School: Kauno r. Raudondvario gimnazija)

Nile Red is a fluorescent, lipophilic organic dye used in fluorescence microscopy for the selective identification of microplastics and in biological research for the localization and quantification of lipids in cells. The classical synthesis method for Nile Red is based on the use of N,N-dimethylformamide (DMF). As restrictions on the use of DMF solvent were approved on 19 November 2021 and are to be applied EU-wide from 2024, it is, therefore, vital to optimize a method based on another solvent that could replace DMF in the synthesis of Nile Red, while maintaining an optimal cost for the compound. The aim of my research is to find an alternative solvent system for the synthesis of Nile Red from naphthol and aminophenol while monitoring HPLC yields of the target compound. For the optimization of the Nile Red synthesis, different classes of organic solvents were selected: acetone, dimethyl sulfoxide, methanol, DMF, and toluene. Nile Red purification was performed using column chromatography and crystallization. For the monitoring of the synthesis, TLC and HPLC-PAD-MS analysis were used. The structure of the final materials was confirmed by means of 1H NMR analysis. The highest HPLC yield for the alternative solvent was obtained in the case of methanol (21 %). Upon further optimization of solvent and reaction time, 2-propanol and ethanol were used. The highest HPLC yield for the Nile Red synthesis was achieved using 2-propanol (44%), ethanol (34%) after 12 h. Nile Red purity of 94 %, suitable for the application in fluorescence microscopy, was achieved. It was demonstrated by the use for staining of cytoplasmic fat droplets, accumulated in cells.

Awards Won:
Third Award of $1,000