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Development of Microreactors and Analytical Methods for Lipid Transformations

by

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Dedication

Bismillahirrahmanirrahim, all praise to the God, the Most Gracious, and the Most Compassionate. I would like to dedicate this special journey to my family, supervisors, friends and loved ones. To Ku and Mama, this is for you.

Abstract

This thesis describes the development of two types of flow-through microreactors containing lipase immobilized onto either a hollow channel support within an optical microstructured fiber capillary (MSF) or onto a silica monolith within a fused silica capillary (SM). The large silica surface area within MSF or SM is suitable for enzyme immobilization. The enzymatic microreactors were used for rapid lipid transformations in small amounts suitable for analytical purposes.

For the SM, the porous monolithic structure was formed based on the sol-gel method involving the reaction of poly(ethylene-glycol) with tetraethyl-orthosilicate at low pH and 40 °C followed by calcination at 200 °C. *Candida antarctica* lipase was immobilized onto both SM and MSF *via* silanization chemistry using glutaraldehyde-linkages. Successful enzyme immobilization was demonstrated by the FTIR spectra at each step of the immobilization process.

The microreactors were tested by performing analytical scale transformations of triacylglycerols by reaction with ethanol. The effects of reaction temperature, flow rate and type of alcohol on the formation of lipid products were investigated. The data from GC and HPLC analyses indicate that the enzymatic MSF-microreactor was regioselective at 50 °C, producing mainly 2-monooleoylglycerol from the transesterification reaction of trioleoylglycerol, at a flow rate of 1 μL/min. Using the SM-microreactor at room temperature, trioleoylglycerol and various vegetable oils (canola, sesame, soybean and refined-bleached-deodorized-palm) were quantitatively transformed into ethyl

esters using flow rates of 0.2-0.5 μL/min. The microreactors were demonstrated to be reusable with minimal loss of activity for >8 runs when operated at room temperature and low flow rates (<1 μL/min). The potential use of the SM-microreactor in automated derivatization for the GC analysis of lipids was described. The SM-microreactor was also used to perform online lipid transformations by coupling it with atmospheric-pressure photoionization-mass spectrometry.

An LC/MS method was developed for in-process monitoring of the epoxidation reactions of triacylglycerols. Reaction intermediates were observed allowing the determination of the time required for full or partial epoxidation of the oil.

Overall, this thesis demonstrates a simple approach to the fabrication of enzymatic microreactors for analytical scale oleochemical transformations. Additionally, suitable methods for analyzing lipid transformations were developed.