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Comparison of Photo-oxidation Reactions in Batch and a New Photosensitizer-Immobilized Microfluidic Device

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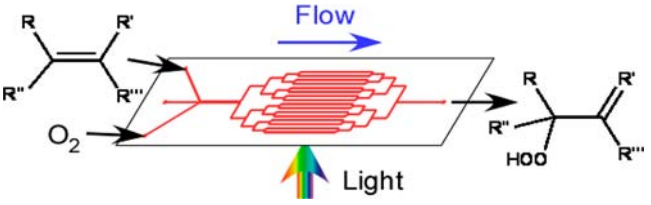
ABSTRACT



A glass microfluidic device has been functionalized with photoactive porphyrins for performing reactions which are mediated by singlet molecular oxygen. The resulting device was used to investigate the photochemical oxidation of cholesterol, α-terpinene, and citronellol under flow conditions, and the results were compared with similar batch reactions.

The use of microreactors for conducting chemical reactions is becoming popular since it enables greater control over mixing and thermal transfer, compared to batch.[1] Additional advantages of microfluidics for photochemical oxidations are shorter path lengths for light penetration and small reaction volumes (nL). This improves safety in handling hazardous reactants,[2] as it ensures only small volumes of solvent are oxygenated at any one time. The number of photochemical reactions conducted within microreactors is growing,[3] as the advantages of increased light penetration are realized. Immobilization of photosensitizers has also been investigated for many decades,[4] as it enables better separation of photosensitizers from products and remaining reactants,[5] thus reducing the need for complicated workup procedures.

There has been much research conducted into the immobilization of porphyrins on solid silica,[4b,6] silica gel,[7] and polymer matrices.[4a,6c,8] There has also been some research into glass-supported photosensitizers,[9] but mostly on micrometer-scale glass beads. Research has also been reported in microphotochemistry, in which the photosensitizer is in solution with the reaction mixture,[2,3,6e,10] while

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solid supported reagents have been shown to be useful in total product synthesis,[11] which would constitute elegant usage for microfluidic applications due to the small scales required. Silica supported porphyrins have also been incorporated into a microfluidic device.[12]

In this research we examine the effect of immobilizing a porphyrin based photosensitizer on the glass walls of a microfluidic device for conducting the photooxidation of three model substrates.

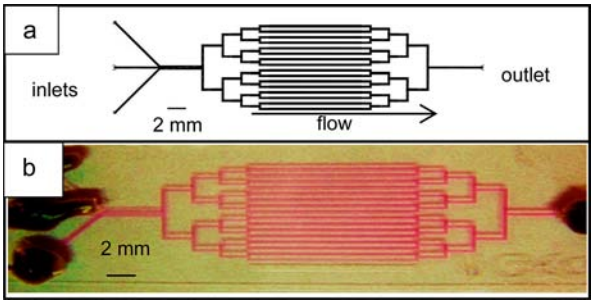


Figure 1. (a) Chip design showing the three inlets and one outlet with the 16 parallel channels; (b) RBITC-immobilized chip.

Silanization is a common procedure for altering the properties of a glass surface, especially on glass in microfluidic devices. (3-Aminopropyl)triethoxysilane (APTES) is a commonly used reagent for silanization reactions, and many groups have used it on glass, both on- and off-chip.[6c,8b,9a,13] The reaction between an amino group and an isothiocyanato group is rapid and can enable a covalent thiourea bond to be formed between the two components. We have previously reported a method for synthesizing AB_3 type porphyrins bearing a single isothiocyanato group.[14] These porphyrins have been shown to be effective photosensitizers.

The chip utilized in the current project was designed with 16 parallel channels originating from three inlets for gas and solutions and one outlet (Figure 1a). With this design it was possible to introduce reagents and oxygen gas directly into the chip and collect product from the outlet.

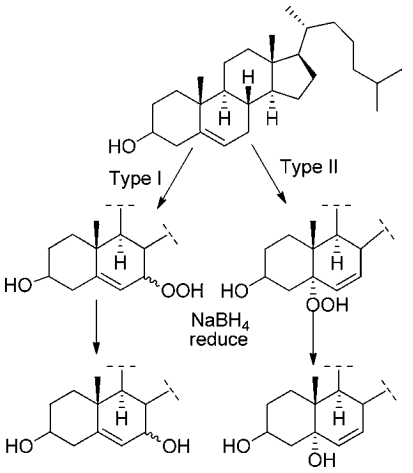
Initially, commercially available rhodamine B isothiocyanate (RBITC) was used to optimize the methodology for immobilizing the porphyrin onto the glass channels. After cleaning the chip with piranha solution (H_2SO_4/H_2O_2 3:1) channels were flushed with 1 mM NaOH solution. The method described in the Supporting Information was found to be optimal in producing a chip in which the rhodamine B was observable, and comprehensive coverage throughout the chip was achieved (Figure 1b).

The use of highly fluorescent rhodamine B to optimize the attachment procedures also allowed possible quenching of excited states to be assessed *in situ*. Therefore, the chip was imaged by fluorescence microscopy after the immobilization procedure (data not shown).

The method that was optimized for RBITC was then transferred to the amino-reactive porphyrin.

The effectiveness of the immobilized porphyrin in producing singlet oxygen was determined through the oxidation of cholesterol to specific, identifiable, products (Scheme 1). The porphyrin immobilized chip was compared with an equivalent batch reaction and also flowing the porphyrin (20 μM) and cholesterol together in solution through the same chip. To ensure comparable results the same isothiocyanato porphyrin that was immobilized on the glass was "capped" at the isothiocyanto group with propylamine prior to the batch and solution experiments.

Scheme 1. Oxidation of Cholesterol by Type I and Type II Photooxidation[a]



[a] Type I photooxidation takes place via radical species to yield 7α/7β-hydroperoxycholesterol. Type II photooxidation takes place via singlet oxygen to yield 5α-hydroperoxycholesterol. These species were then reduced with sodium borohydride to give the stable hydroxycholesterol products.

The porphyrin concentration of 20 μM was selected as this gave a good percentage conversion while allowing effective transmission of light through the solution. This compares with approximately 0.332 nmol of porphyrin attached to the glass channels, assuming an average coverage of 2 porphyrins per nm^2.[15]

Analysis of the resulting solutions was conducted using HPLC (UV detection; λ = 215 nm) as described in the electronic Supporting Information. It was found that although the expected product for singlet oxygen mediated peroxidation of cholesterol would be the 5α-hydroperoxycholesterol, in both the batch and microfluidic regime the 7α/7β-hydroperoxy and 6α/6β-hydroperoxy cholesterol

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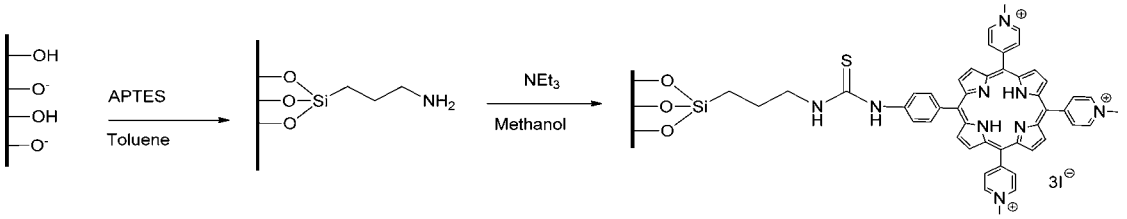
Scheme 2. Immobilization of Porphyrin on to Glass Channel Walls[a]



[a] The porphyrin was immobilized by the reaction of the isothiocyanate group with an amino group introduced by silanization with (3-aminopropyl)-triethoxysilane (APTES).

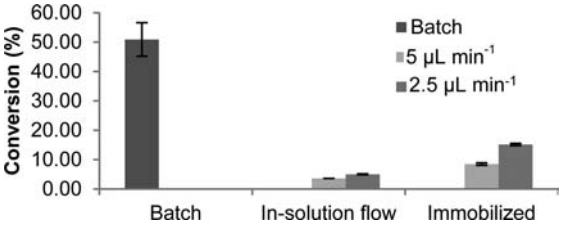


Figure 2. Graph showing the oxidation of cholesterol in batch and on-chip with porphyrin in solution and immobilized on the glass channel walls for two flow rates.

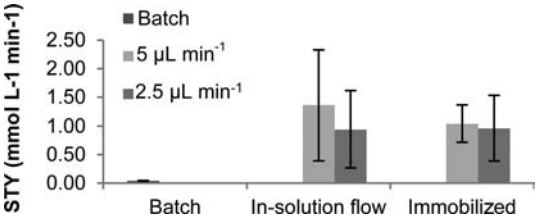


Figure 3. STY graph for the oxidation of cholesterol in batch and on-chip.

were also observed. This suggests that Type I reactions are also occurring, or that the molecule is rearranging over time. The results were quantified by the area under the curve of the oxidized cholesterol products and the remaining unreacted cholesterol peak. The peaks were verified by HPLC-MS to confirm that they were the expected oxidized cholesterol products.

The results are presented in Figure 2 and show that the immobilized porphyrin (Scheme 2) was capable of photo-oxidizing cholesterol. In addition it also appeared that the immobilized photosensitizer was more effective at oxidation of cholesterol than a comparable flow experiment on-chip, with the porphyrin in solution. As the lifetime of $^1O_2$ is short it is likely that diffusion is low even when the photosensitizer is in solution, possibly accounting for these results. Neither of the flow reactions was capable of producing yields of oxidized cholesterol close to those obtained in the batch reaction. However, the batch reaction was conducted for 60 min, in comparison to a residence time of approximately 30 s for the flow reaction on-chip.

A method of determining the efficiency of these results which takes into consideration the reaction volume and duration has been previously reported[16] and expresses this as space time yield (STY)

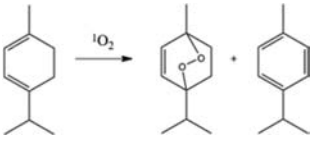
$$\mathrm{STY} = n/(V_R t)$$

with n = amount of substance converted (mmol), V_R = reactor volume (L), and t = irradiation time (s).

This equation allows a better estimation of the efficiency of the reaction, especially for reactions that have not gone to completion. For the cholesterol experiment the results show (Figure 3) that the microfluidic regime has an increased efficiency in producing singlet oxygen.

In order to explore the diversity of this method other representative oxidation reactions involving singlet oxygen were tested. α-Terpinene is known to undergo a [4 + 2] electrocyclic reaction with singlet oxygen to produce ascaridole (Scheme 3). The oxidation of citronellol is another example of an ene-type oxidation mediated by singlet oxygen. The reaction is very useful in the perfume industry, as it enables the production of rose oxide (Scheme 4). Consequently, these substrates were selected to broaden the scope of this work.

Scheme 3. Oxidation of α-Terpinene by Singlet Oxygen via the Diels–Alder [4 + 2] Reaction to Yield the Oxidation Product Ascaridole and the Byproduct *p*-Cymene



As before, batch and flow photo-oxidation reactions were compared. GC-MS was used to analyze the results of the α-terpinene reaction and to identify products. It was found that for the batch reaction the α-terpinene produced

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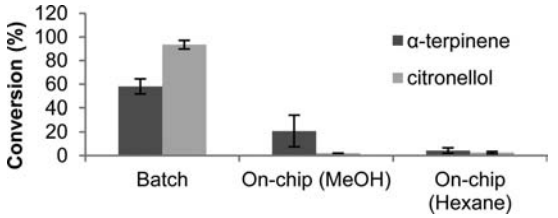
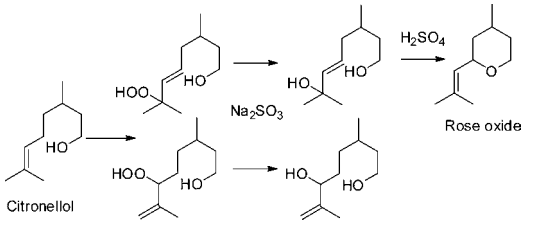


Figure 4. Graph showing the oxidation of α-terpinene in batch and on porphyrin immobilized chip in polar and nonpolar solvents.

Scheme 4. Oxidation of Citronellol by Singlet Oxygen via the Ene Reaction[a]



[a] The resulting hydroperoxy products can be reduced to the hydroxy products, and the 2-hydroxy product can be made into rose oxide which is used in the perfume industry.

additional oxidation products such as eucalyptol and other oxabicyclo structures in the early stages of the photooxidation. However, after further irradiation, ascaridole was formed in high yields (Figure 4). For routine analysis of the citronellol reaction, RP-HPLC with UV detection (λ = 215 nm) was used. In order to verify the products, the combined mixture from the batch reactions was purified using column chromatography and analyzed by NMR to match literature values.[17]

The yields for α-terpinene and citronellol in batch were high; as expected, citronellol oxidation proceeded almost to completion. The on-chip yields for α-terpinene in methanol were higher than in hexane. The on-chip yields for citronellol were very similar to one another and both low.

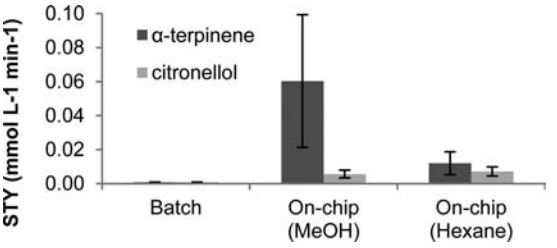


Figure 5. STY graph for α-terpinene and citronellol. Despite the high yield achieved in batch for both reactions the STY is very low. The STY for on-chip reactions was much higher for α-terpinene but still relatively low for citronellol.

The STYs for α-terpinene and citronellol reactions are shown in Figure 5. The α-terpinene reaction shows a marked increase in STY on-chip as compared to the batch; however, as expected, the citronellol STY is only slightly better than that for batch.

In summary, we have shown that porphyrin immobilized on the glass channels of a microfluidic chip is capable of producing singlet oxygen with high efficiency and with no photobleaching within the time period of the experiment. Although the overall yields for these reactions under flow conditions are modest, the advantages of producing oxidation products on extremely small samples, with improved efficiency and purity, could be of use in identifying new products in the area of natural products where sample sizes are often limited and finite.

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Supporting Information Available. Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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