IMMOBILIZED ENZYME CATALYZED POLYMERIZATION REACTIONS IN MICROREACTORS[a]

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Introduction

Synthesis of polymers using enzymes as a catalyst provides an alternative way to reduce the use of toxic metal catalysts and to enable milder processing conditions.[1,2] However, to use enzyme catalyzed systems in commercial manufacturing, all reaction parameters need to be identified and then optimized through systematic, rigorous and quantitative characterization techniques. In most of the previous studies on enzymatic catalytic polymerization, reactions were performed in lab scale batch reactors, where enzymes and reactants were mixed (often in the presence of an organic media) and time evolution of monomer conversion and relative molecular mass change was captured *ex situ*. Since enzymes are not typically soluble in the organic phase, and economic viability of these methods depends on recycling enzymes, they are often immobilized on inert beads or stabilized with a surfactant system. Although these studies are important to understand the reaction, many questions remain unanswered, such as the effect of system geometry, mass transfer processes, and flow. In addition, the batch reactors are not inherently attractive for large scale industrial processes and are not suitable for real time measurements to capture time dependent changes in the reacting systems. As an alternative, in this study we have used a Lab-on-a-Chip approach or microreactor technologies to develop a model measurement platform. Our goal is to use these high throughput techniques for measuring reaction variables and resulting polymer characteristics in enzyme catalyzed reactions. Microreactor technologies enable improved safety, selectivity and yield in a range of chemical reactions.[3] We have developed this approach using a widely investigated system, ring opening polymerization of ε-caprolactone to form polycaprolactone by using *Candida antartica* Lipase B (CAL B) as a catalyst (**Figure 1**).[4]
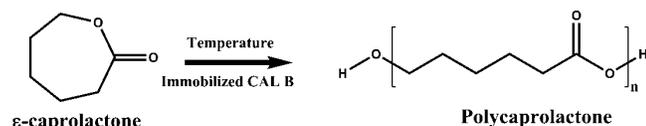
Figure 1. Ring opening polymerization of ε-caprolactone to polycaprolactone.

Experimental

The experimental set up is shown in **Figure 2**. The chip (microreactor) is made of aluminum consisting of embossed channels with width and depth of 2 mm and 1 mm, respectively. For the present study the channel length is 26 cm. The channels are covered with Kapton[5] film using Resinlab epoxy
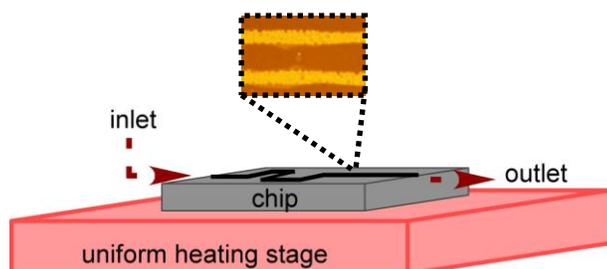
Figure 2. Schematic of typical microreactor. Inset is image of aluminum channel filled with enzyme immobilized beads.

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adhesive. The channels are filled with commercially available Novozyme beads. These beads (≈400 μm diameter) are crosslinked polymethylmethacrylate (PMMA) support (Lewatit) with immobilized CAL B. The amount of catalyst loaded in the present system is 200 mg and the void fraction of the system is ≈0.5. We introduce a 2:1 mixture of toluene and ε-caprolactone in the microreactor with a flow rate of 50 μL/min, which corresponds to a residence time of ≈5 min. The microreactor was placed on a uniform heating stage to maintain the experimental temperature at 55 °C. The temperature inside the microchannel was occasionally measured by inserting a thermocouple. The product streams from the microreactor were collected and have been characterized using gel permeation chromatography (GPC), Raman spectroscopy, and nuclear magnetic resonance (NMR). The GPC measurements were performed using toluene as a solvent and polystyrene as a standard. The monomer conversion was measured using Raman spectroscopy[6] and in this technique we capture the disappearance of characteristic ε-caprolactone peaks, 694 cm^{-1} and 732 cm^{-1}, as it is converted to polycaprolactone. Peaks at 694 cm^{-1} and 732 cm^{-1} correspond to anti-symmetric ring stretching and ring breathing of ε-caprolactone, respectively. Since polycaprolactone is a linear polymer, these peaks are absent for polycaprolactone.

Results and Discussion

Figure 3 displays a critical section of the Raman spectra obtained for both the feed and product streams. The 694 cm^{-1} and 732 cm^{-1} peaks for ε-caprolactone in the inlet stream disappear in the product stream indicating that conversion of ε-caprolactone was > 90%. Such finding was also verified using NMR.
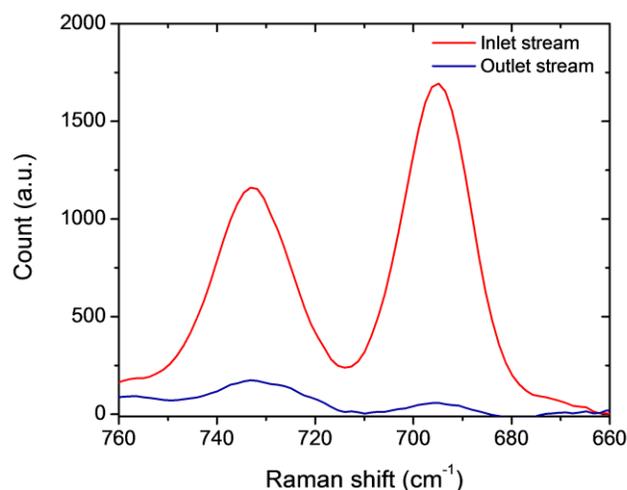
Figure 3. Typical Raman spectra for inlet and outlet streams.

The GPC trace for the product stream is shown in **Figure 4**. Also, shown is the GPC trace for a sample prepared using a batch reactor. The reaction time in the batch reactor was 45 min and the corresponding conversion of ε-caprolactone was > 90%. The estimated number average relative molecular mass (M_n) of the samples obtained from microreactor is 11500 g/mol and that from batch reactor is 7200 g/mol (polystyrene standard). Although similar conversion have been achieved in both batch and micro reactors, one striking difference between batch and micro reactors is the reaction time, 45 min in batch reactor *vs.* 5 min in microreactor. As reported in the literature, in the batch reactors, the time required to reach high conversion can vary from an hour to a few days, depending upon the catalyst used.[2,7] The one order lower reaction time in the microreactor is likely due to high catalyst surface area to low reactor volume ratio, which increased by at least 20 times in microreactors compared to that of batch reactors. Such high surface area cannot typically be achieved in the batch reactors. Another potential advantage in the microreactor is the uniform temperature that the materials are subjected to, which could not be easily achieved in the traditional batch reactors. Results from batch and microreactor are summarized in **Table 1**.

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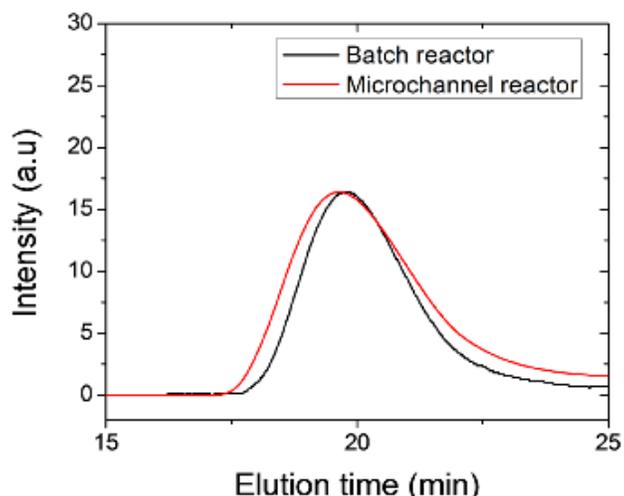
Figure 4: GPC traces of products from batch and microreactor.

Table 1. Comparison of batch and microreactor*

	Reaction/residence time	M_n	M_w	PDI
Microreactor	5 min	11500±2600	29000±6500	2.5
Batch reactor	45 min	7200±1200	15000±1500	2.1

*The errors indicate one standard uncertainty based on measurements on 5 different samples. M_n is number average relative molecular mass, M_w is weight average relative molecular mass, and PDI is polydispersity index.

In this first generation device, little effort was made to dry the system or probe the effects of temperature and flow conditions more systematically. On-going work includes the use of this device to obtain accurate and reliable apparent rate data under a variety of conditions to better understand the many subtle differences between continuous reactions in a micro-environment as opposed to traditional batch systems and to begin probing the extent of contol possible in the system.

Conclusions
In summary, we have developed a microreactor platform to study enzyme catalyzed reactions. The system was developed around the comparatively well-understood lipase-catalyzed polymerization of caprolactone. The monomer conversion in microreactors is faster than that observed in the batch reactors. This platform can be scale up in industry to obtain enzyme catalyzed polymers in continuous mode. We are further studying the reaction kinetics of these systems

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5. Certain commercial equipment, instruments, or materials are identified in this paper in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.
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