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A microreactor with phase-change microvalves for batch chemical synthesis at high temperatures and pressures†

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We present a simple microreactor with dimethyl sulfoxide (DMSO) phase-change valves suitable for performing batch organic chemistry under high temperature and pressure conditions. As a proof of principle, we demonstrate a radiofluorination reaction important in the synthesis of [^{18}F]FAC, a new positron emission tomography biomarker for immune system monitoring and prediction of chemotherapy response. We achieved high radioactivity recovery (97 ± 1%, n = 3) and conversion efficiency (83 ± 1%, n = 3), comparable to that achieved with macroscale systems, but with a volume 30× smaller. This platform overcomes the limitations of previously reported phase-change valves in terms of compatibility with organic chemistry, and extends the range of reaction conditions for carrying out harsh batch chemistry at the microscale.

Introduction

Performing chemical reactions in a microscale format has significant advantages for many applications.[1] Batch microreactors, as opposed to continuous flow microreactors, provide the ability to perform reactions in extremely small total volumes. They are especially suited for working with scarce reagents, such as natural products or isolated proteins, or for producing short-lived radiolabeled molecular imaging probes for positron emission tomography (PET),[2] where it is desirable to work with extremely low mass quantities. Another advantage of batch microreactors is the ability to implement multi-step processes in a single reactor, simplifying the device and minimizing transfer losses.

Though several microfluidic batch reaction platforms have been reported in the past decade[3–7] they tend to be limited to more mild reaction conditions (*i.e.*, lower temperatures and pressures) than their continuous flow counterparts. Handling high pressures is important for many applications, however. To ensure fast kinetics and high yields, the radiosynthesis of short-lived imaging probes for PET is often performed in a sealed vessel under super-heated conditions, resulting in the generation of significant solvent vapor pressure. For example, the probe 1-(2′-deoxy-2′-[^{18}F]fluoroarabinofuranosyl)cytosine ([^{18}F]FAC)[8] and its analogs require the containment of the vapor pressures of solvents well in excess of 100 psi.[9,10] Pressurized conditions may also be important more generally in organic chemistry as pressure can accelerate reactions that are accompanied by a decrease in volume and shift the equilibria toward the side of the products.[11] In some other cases, high pressures are generated by the formation of gaseous productions.

Current batch microreactors are typically incompatible with harsh conditions due to several limitations. Pressure compatibility of poly(dimethylsiloxane) (PDMS)-based microreactors[3,4] is severely restricted by the inter-layer bonding strength as well as by vapor-permeability of the PDMS itself; furthermore, PDMS is incompatible with many solvents and reagents. Digital microfluidic chips,[5,6] made of materials including glass and fluoropolymers, have emerged as a more chemically inert batch reaction platform, but these chips have an open structure and cannot perform pressurized processes unless the entire chip is placed inside a pressure chamber.[12] Recently, using a closed geometry, and rigid, non-permeable polymers, Lebedev *et al.*[7] recently demonstrated a 50 μL batch reactor for multi-step reactions that can withstand pressures up to 300 psi; however, the device assembly and fabrication are complex and the large size and positioning of the off-chip valve actuators increases the overall footprint of the microreactor system and risk of mechanical failure.

Microvalve technologies reported capable of withstanding high pressure can be potentially employed to develop batch microreactors that do not suffer the above limitations. Although various microvalves have been reported,[13–20] very few of them have been extensively developed for practical use. MEMS-based mechanical microvalves, where deformable

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membranes are coupled to magnetic, electric, piezoelectric, or thermal actuation methods, generally have low maximum pressures with a couple of exceptions.[13] In addition, these valves generally have a relatively complicated structure and require multi-step fabrication including multi-layer alignment, bonding and assembly, resulting in the difficult integration with an overall microfluidic reaction platform. Valves based on mobile free-standing polymer elements have been demonstrated to withstand pressures up to 4500 psi,[14] however, the additional steps of monomer filling and patterned *in situ* polymerization complicate the device fabrication. In contrast, phase-change microvalves comprise a very straightforward and convenient alternative approach, relying on reversible solidification of liquid in the channel itself to form a solid plug to obstruct the fluid path. Impressive reports demonstrate the capability of to withstand up to 1450 psi, without the need for complex fabrication or moving parts. Since the first report,[15] a variety of materials including water,[16] paraffin,[17] hydrogel,[18] polymer monolith[19] and metal alloys[20] have been chosen as the actuation liquid in these valves, with phase transitions triggered thermally, chemically or optically. Thermally-actuated valves based on water or paraffin have been used in biochemistry for performing polymerase chain reaction (PCR)[16,17] and those with metal alloys have been used for gas sampling applications.[20] However, limited by material incompatibility, none of them would be suitable for radiochemistry or more general chemical reactions. For example, paraffin and many polymers are soluble in commonly used organic solvents, which could lead to contamination of the reaction mixture and instability of the valves; water and hydrogel materials present significant concerns for water-sensitive reagents and reactions; and metals are likely to be problematic in reactions involving strong acids and bases.

Using a new phase-change material, dimethyl sulfoxide (DMSO), we extend the application of phase-change valves to high-pressure organic chemistry in batch microreactors to address the limitation of current phase-change valves. In the field of radiochemistry, DMSO is a commonly used solvent and is thus intrinsically favorable as a phase-change material. The solvent compatibility allows direct translation of a known chemical reaction onto a microfluidic platform, without the need to change the chemistry to avoid the risk obtaining sub-optimal yield due to contamination. Physically, DMSO has a relatively high freezing point (18.9 °C) making it easier to freeze than water and many other organic solvents. As a demonstration of this microreaction platform, we performed the high-temperature and pressure radiofluorination step in the synthesis of the molecular probe [^{18}F]FAC. Our new platform, based on a simple architecture, broadens the range of reaction conditions in batch microreactors, and enables increased diversity of microscale batch chemistry applications.

Experimental

The batch reactor (Fig. 1) was implemented with capillary tubing (polytetrafluoroethylene (PTFE), OD: 0.030″, ID: 0.012″, Cole-Parmer). The central portion of the capillary (43 cm long and 31 μL in volume), designed to contain the reactant mixture, was coiled and sandwiched between two heating blocks (each 5.0 × 5.0 × 1.2 cm); and the distal portions (each 4.4 cm long and 3.2 μL in volume), designed to contain slugs of the phase-change liquid DMSO (>99%, from Sigma-Aldrich, colored by Nile Red (Sigma-Aldrich) for clear visualization), were coiled and mounted on the cold side of a thermoelectric Peltier cooler (CP854388, CUI Inc.). Coiling turns were packed as flat as possible and held in place by Kapton® tape (Kapton tape Inc.) with thermal paste to ensure uniform heating along the length of the capillary. To improve cooling, the hot side of the Peltier was affixed to an aluminum block with an internal flow path connected to a recirculating liquid-cooling system (Freezone Elite FZ-1003, CoolIT Systems). It took ~1 min after activation of the Peltier and cooling system to cool the top surface of the Peltier from room temperature down to a steady state value of −19 °C. This temperature is sufficient to rapidly solidify the two DMSO slugs, to seal the contents in the central region of the capillary. To provide heating to activate chemical reactions in this central region, each heating block was equipped with a heater (C1E13, Waltlow), thermocouple (K type, CHAL-002, Omega) and dedicated temperature controller (CN7523, Omega). The entire experimental setup was operated inside a lead hot cell to provide radiation shielding.

The heating and cooling blocks were positioned 1.5 cm apart, with the volume of gradient region between heating

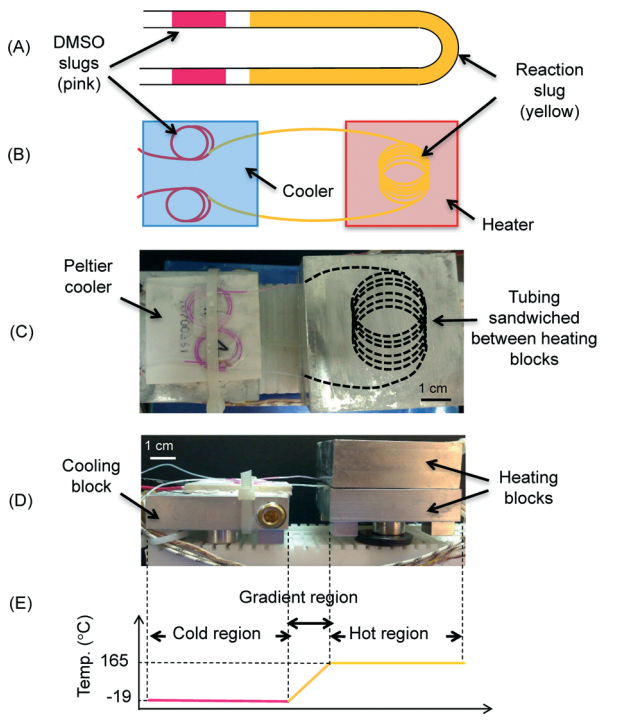

Fig. 1 (A) Simplified schematic of batch microreactor. (B) Schematic showing actual capillary configuration on the heating and cooling blocks. (C) Top view and (D) side view photographs of the reaction platform. (E) Estimated temperature profile along the capillary under the assumption of one-dimensional linear heat transfer model.

and cooling blocks as ~2.2 μL. On one side, the gradient region needs to be long enough so that DMSO freezing was not compromised when the capillary contained in the reaction region is heated. On the other side, because the liquid inside the capillary in the gradient regions is not uniformly heated, it is desirable to minimize the ratio of this volume to the volume in the reaction region. In our gradient region design, a trade-off was made between these two considerations: the volume of the gradient region accounts for ~7% of the total reaction volume whose effect on the reaction performance is discussion in the next section, and the effect of heat convection on valve performance is included in ESI.† The volume in the reaction region could easily be scaled up or down by changing the capillary length (*i.e.* number of coiling turns) for other potential applications.

The steps to perform a reaction inside the microreactor are illustrated in Fig. 2. Slugs of DMSO (3.2 μL), reactant mixture (31 μL), and DMSO (3.2 μL) are aspirated into the capillary by a computer-controlled syringe pump (PSD/4, Hamilton). To minimize cross-contamination between DMSO slugs and the reactant mixture, small air gaps (0.3 cm, 0.2 μL) were introduced between these liquids. The reaction mixture is then heated to the desired temperature for the desired period of time. Upon completion, the heater was turned off and the capillary was lifted away from the heater, allowing the reaction chamber to cool to room temperature. Next, the cooler was deactivated to melt the DMSO and open the valves. Finally, the product is sequentially collected from the capillary into three collection vials (separate vials for DMSO slugs and reaction mixture) for subsequent analysis.

As a demonstration of a reaction involving high pressure, the radiofluorination step of the synthesis of [^{18}F]FAC (Fig. 3) was performed. High temperature and pressure are essential for obtaining high yield. First, [^{18}F]fluoride (5 μL of cyclotron-bombarded [^{18}O]H_2O containing 3–4 mCi) in a solution of K_2CO_3 (0.71 mg mL^{-1}) and $K_{2.2.2}$ (7.14 mg mL^{-1}) in a 1.4 mL mixture of acetonitrile (MeCN) and H_2O (0.4 : 1 v/v) was evaporated to dryness at 110 °C in a 5 mL vial. This was followed by three cycles of azeotropic distillation, each using 0.5 mL MeCN, evaporated at 110 °C, to form an anhydrous residue of the [^{18}F]KF/$K_{2.2.2}$ complex. K_2CO_3, $K_{2.2.2}$, and anhydrous MeCN were purchased from Sigma Aldrich and used as received. After cooling the complex to room temperature, 350 μL of 2-*O*-(trifluoromethylsulfonyl)-1,3,5-tri-*O*-benzoyl-*α*-D-ribofuranose (ABX Advanced Biochemical Compounds) in MeCN (14.3 mg mL^{-1}) was added and mixed by bubbling nitrogen. The radiofluorination reaction was then performed in the batch microreactor as described above, under the reaction condition of 165 °C for 15 min. Following the reaction, the DMSO slugs and reactant mixture were separately collected. Samples were analyzed by radio-thin-layer chromatography (radio-TLC) in a mobile phase of 95 : 5 v/v MeCN/H_2O and the conversion efficiency calculated from the integrated area under two peaks in the resulting chromatograms (obtained from mini-GITA radio-TLC scanner, Raytest). One peak represented the unreacted [^{18}F]fluoride ion (retention factor R_f = 0.0), and the second corresponded to the desired fluorinated product (R_f = 0.97). The radioactivity in each vial was measured using a calibrated dose calibrator (CRC-25PET, Capintec). The initial amount of radioactivity loaded into the capillary was calculated as the difference in radioactivity

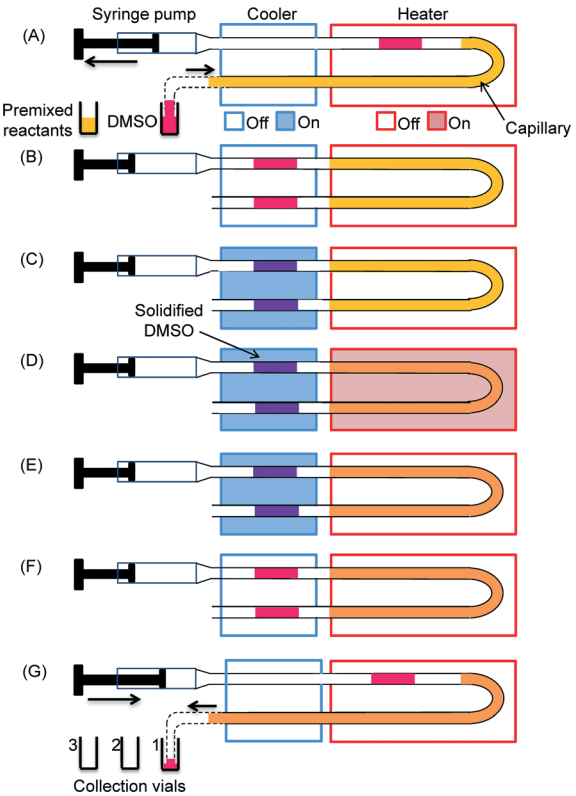

Fig. 2 Schematic of batch microreactor operation. (A) DMSO, air, and reagent slugs are aspirated by the syringe pump; (B) slugs are moved to designated locations in the tubing; (C) cooler is turned on to close valves, *i.e.*, solidify DMSO (purple); (D) heater is activated to perform reaction; (E) heater is deactivated to cool reaction mixture and reduce pressure; (F) cooler is turned off to open DMSO valves (pink); (G) the samples are pumped out into collection vials for subsequent analysis.

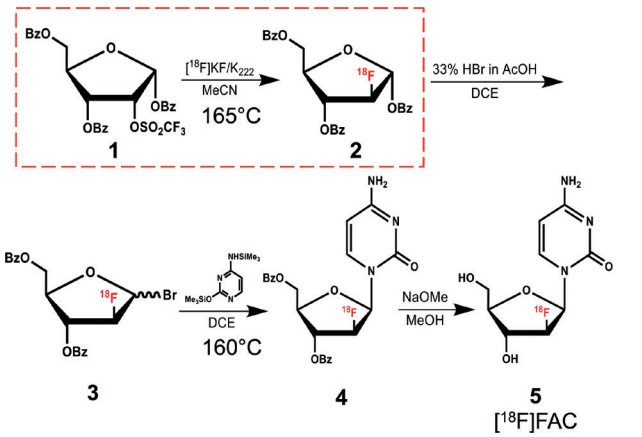

Fig. 3 [^{18}F]FAC synthesis scheme. The radiofluorination step (dashed box) from precursor **1** to the fluorinated sugar **2** was chosen as a demonstration reaction.

of the premixed reagent vial before and after loading. The total radioactivity after the reaction is the sum of radioactivity of samples in all collection vials. All radioactivity measurements were decay-corrected to the start time of the experiment. As a control experiment, the same procedure was followed except that the cooler was not activated to freeze the DMSO. The capillary was disconnected from syringe pump after loading and its two ends were inserted into two empty collection vials. Samples collected were measured for radioactivity and their composition analyzed by radio-TLC.

Results and discussion

Our DMSO phase-change microvalves have been characterized in terms of maximum withstanding pressure and response time. Based on our investigation of the effects of slug length, capillary diameter, and curvature of the capillary "turns", on valve withstanding pressure (Fig. S1 and ESI†), a 0.012″ ID capillary with DMSO slug of length 4.4 cm was chosen for our demonstration reaction. The maximum withstanding pressure of DMSO valves with this particular geometry is characterized to be at least 450 psig, governed by the limit of our pressure source. This pressure is significantly higher than 121 psi – the anticipated pressure during the reaction estimated according to the vapor pressure of the reaction solvent MeCN at the reaction temperature 165 °C. Freezing of the DMSO slugs in the capillary was found to occur within 2 s of contacting the capillary with the cooling block pre-cooled to −19 °C. The DMSO colored by Nile Red turned from pink to dark purple as the phase changed from liquid to solid. The valve re-opened in 25 s after the Peltier was deactivated. These response times are more than adequate for most chemical applications, including the synthesis of short-lived radioisotopes which require fast operations. With optimization of the geometry and the heating and cooling systems, response times could likely be decreased further. Response times down to 80 ms have been reported in other configurations.[16] As in the reaction the batch sealed by two DMSO valves is heated (Fig. 1), studies to examine the effect on valve stability of heat conduction from heaters to DMSO slugs is included in ESI.† The DMSO slugs remained frozen for 15 min and no leakage was observed, when the temperature of the heater region was elevated and kept at up to 200 °C, ensuring that the valve performance is more than sufficient for the reaction at 165 °C.

As a demonstration of the utility of a batch microreactor capable of withstanding high pressures, the radiofluorination step in the synthesis of [^{18}F]FAC, a new PET probe for immune system monitoring and prediction of chemotherapy response,[4] was performed. The percentage of radioactivity recovered after each experiment was nearly ideal (97 ± 1%, n = 3), consistent with the observation that no leaking was visible during the experiments. Radio-TLC analysis of the collected samples showed the conversion of [^{18}F]fluoride ion to the fluorinated product to be 83 ± 1% (n = 3), which is comparable to typical results attained using a macroscale radiosynthesizer.[10] A representative radio-TLC chromatogram is shown in Fig. 4. Taking into account the small volume fraction (~7%) contained within the temperature gradient region, the conversion efficiency is expected to be slightly higher if the microreactor design was further optimized, or if it was implemented in a microfluidic chip with tiny thermoelectric junctions[16] where the fraction of gradient region is reduced. The high conversion efficiency also suggests that the residue DMSO left behind into the reactant mixture during sample loading and collection, does not adversely affect the reaction, and/or the level of this contamination is low. The observation that only 3 ± 2% (n = 3) of the total recovered radioactivity present in the collection vials containing the DMSO slugs, verifies the overall cross-contamination at a low level. Besides the contamination occurring during sample loading and collection and a possible trace amount of reaction mixture redistributed through the air gaps by evaporation/condensation during the reaction, the main contamination comes from the imprecise nature of the manual operations involved in DMSO–crude product–DMSO sequential sample collection. An automated sample collection system employing liquid sensors could improve the accuracy and reproducibility of sample collection. In future applications that are more sensitive to cross-contamination, it may also be possible to place intervening "washing" slugs between the reactant mixture and the DMSO slugs.[21]

To confirm the need for high-pressure valves in the batch reactor for our particular model reaction, the reaction was repeated without first freezing the DMSO valves. In three separate attempts, it was observed during heating that the reaction mixture "burst" and pushed all slugs, into the collection vials at the two ends of the capillary. Radioactivity measurement and radio-TLC showed 98 ± 2% (n = 3) of the radioactivity initially loaded into the capillary was lost into these collection vials and conversion efficiency was 0% (n = 3). These results suggest the importance of properly closed DMSO valves to contain the high pressure generated during the batch reaction process. In the multi-step synthesis of PET probes, the radiofluorination step is often the most difficult, requiring the highest temperatures and pressures. One could envision performing additional reaction steps in this batch

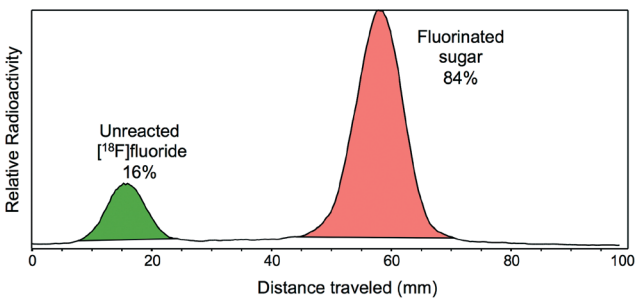

Fig. 4 Representative radio-TLC analysis of crude product of radiofluorination reaction demonstrated in capillary reactor with phase-change valves.

microreaction platform, and integrating with microscale technologies for upstream fluoride drying and postreaction purification for a fully-automated platform to synthesize ^{18}F-labeled molecular probes such as [^{18}F]FAC and its analogs.

This platform could also be suitable for high-throughput screening or combinatorial chemistry applications where high temperatures and pressures are needed. Though we have shown a single microreactor with DMSO phase-change valves implemented in a section of a capillary, this concept could readily be extended to larger numbers of reactors and phase-change slugs by leveraging significant advances in spatially-defined slug-based systems for high-throughput screening.[22] Despite our implementation of the microreaction platform in a capillary for simplicity, with substantially more effort, we see no technical hurdles in extending this work into a microfluidic chip, as several previous efforts have successfully implemented phase-change valves in a microchip format.[16] Because phase-change valves themselves require no fabrication, tremendous flexibility of chip material and geometry can be considered to meet the needs of different applications. With the possibility of multiple individually-addressable phase-change valves on a single chip,[16] sophisticated liquid handling functionality could be integrated on-chip, simplifying the preparation and collection of slugs and reducing the size and cost of the overall microreaction platform. Implementation in a microfluidic chip may also enabled geometries with improved heating and mixing efficiency. It should be appreciated, however, that batch-type microreactors have inherently inferior mixing and thermal performance compared to continuous flow and continuous droplet microreactors and thus may be less suitable for certain types of very fast reactions.

Conclusions

We have developed a batch microreactor using phase-change valves for high temperature and pressure chemical reactions. With the use of DMSO as a new phase-change material, this platform extends the application of phase-change valves from applications in biology to applications in organic synthesis involving harsh conditions or moisture-sensitive reagents. The ability for this simple microreactor to hold high pressures enables batch reactions to be performed under a wider range of conditions (temperatures, pressures, reagent diversity) than possible in previous batch devices.

As an example reaction, we successfully demonstrated the radiofluorination step in the multi-step synthesis of [^{18}F]FAC. The synthesis was performed with high conversion efficiency comparable to what can be typically achieved under sealed, pressurized conditions at the macroscale. This platform would be suitable for performing many other batch chemical reactions at the microscale, especially those that involve high pressures due to high vapor pressure under superheated conditions, or due to production of gaseous products during reactions.

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