MULTI-STEP ORGANIC SYNTHESIS OF FOUR DIFFERENT MOLECULAR PROBES IN DIGITAL MICROFLUIDIC DEVICES

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ABSTRACT

Digital microfluidic devices are a versatile platform for batch chemical synthesis at the microscale. They are compatible with a wide range of chemicals and temperatures, their open structure facilitates evaporations that enable solvent exchange processes which are critical in multi-step reaction schemes, and the use of small volumes permits optimizations that can lead to reduced synthesis time. These properties and advantages are especially suitable for the multi-step synthesis of molecular imaging tracers for positron emission tomography (PET). We demonstrate the reliable multi-step synthesis of four compounds labeled with the short-lived radioisotope fluorine-18.

KEYWORDS

Electrowetting on dielectric (EWOD), Radiosynthesis, Multi-step synthesis, Organic chemistry, Digital microfluidics

INTRODUCTION

Manipulating droplets in digital microfluidic devices presents a flexible new avenue for micro-chemical batch synthesis [1–3]. The chemically-inert and temperature-stable materials used to fabricate digital microfluidic devices enables compatibility with a wide range of reagents and reaction conditions, and the open sides of the chip facilitate solvent evaporation. Digital microfluidic devices with droplet manipulation electrodes and integrated heaters can perform all of the processes needed for multi-step organic synthesis including reagent mixing, reactions at ambient or elevated temperatures, as well as evaporations and solvent exchange. Solvent exchange is necessary in most multi-step syntheses to ensure that each reaction step is performed in the most favorable solvent. While clever schemes for solvent exchange with immiscible [4] or miscible [5] solvents in continuous flow systems have been demonstrated, digital microfluidics enables solvent exchange to be performed by evaporation in a manner that it is intuitively familiar to chemists. The initial solvent is evaporated by heating the droplet until only a dry residue remains, followed by introducing a droplet of the new solvent to re-dissolve the residue. In addition to the possibility of performing sophisticated chemical transformations on-chip, approaches have been developed to perform intermediate or final purification steps [6-7] on digital microfluidics.

To demonstrate the versatility and reliability of the micro-chemical synthesis platform, the effectiveness of on-chip solvent exchange, and advantages of performing reactions in small volumes, we produced several short-lived compounds with syntheses of varying complexity.

EXPERIMENTAL

The electrowetting-on-dielectric (EWOD) chemical reaction chip is illustrated in Figure 1. Reagents are transported by sequential activation of EWOD electrodes from any of several loading sites to the central heater where mixing, reactions, and evaporations take place. When evaporating solvent in the EWOD chip, the vapor diffuses away from the heater site and recondenses in nearby, cooler regions of the chip (Figure 2). To ensure reliable removal of solvent, we use an inert gas flow to more efficiently transport vapor away from the heater site (Figure 3).

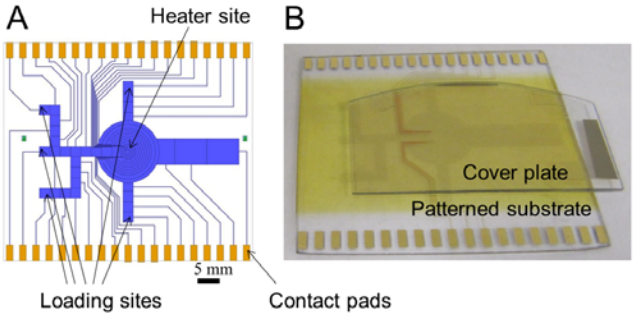

Figure 1: Digital micro-chemical synthesis chip. (A) Design of patterned electrode layer. (B) Photograph of assembled EWOD chip with cover plate and patterned substrate separated by spacers.

The synthesis of fluorine-18-labeled tracers [1,8] requires an initial complexation reaction with K_2CO_3 and Kryptofix-222 ($K_{2.2.2}$) or tetrabutylammonium bicarbonate, followed by an evaporative drying process to eliminate the water from the [^{18}F]fluoride ion supplied from a cyclotron, and thus create an organic solvent-soluble form. Following the drying step, a precursor solution is loaded, mixed by EWOD actuation and then heated to redissolve and react with the activated/dried [^{18}F]fluoride complex residue in the fluorination step. This reaction is typically

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extremely water-sensitive and thus it is critical to eliminate residual water during the initial water removal process. Droplets of volatile solvents such as acetonitrile (MeCN) rapidly evaporate when heated, making it difficult to perform reactions above the solvent boiling point as is typically done at the macroscale where the reactor vessel is sealed. To overcome this limitation, fluorinations were performed in mixtures (typically 4:1) of the desired solvent with a compatible higher boiling solvent such as dimethylsulfoxide (DMSO). Rapid evaporation of the more volatile solvent component leads to an increasingly concentrated reaction mixture eventually composed of only DMSO. Though an excellent solvent for many fluorination reactions, DMSO is frequently avoided in macroscale radiosynthesis because its low volatility makes it difficult to remove in a short amount of time; however, due to the small volumes used in the chip, the DMSO partially evaporates by the end of the fluorination reaction to sub-µL volume that retains solvation of reactants but remains below the allowed limits for injection in animals or humans. This general procedure was followed to perform [^{18}F]fluoride drying and fluorination reaction of several syntheses (Figure 4): (a) [^{18}F]fallypride (1-step), (b) 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG) (2-steps), (c) [^{18}F]fluorothymidine ([^{18}F]FLT) (2-steps), and (d) N-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) (3-steps).

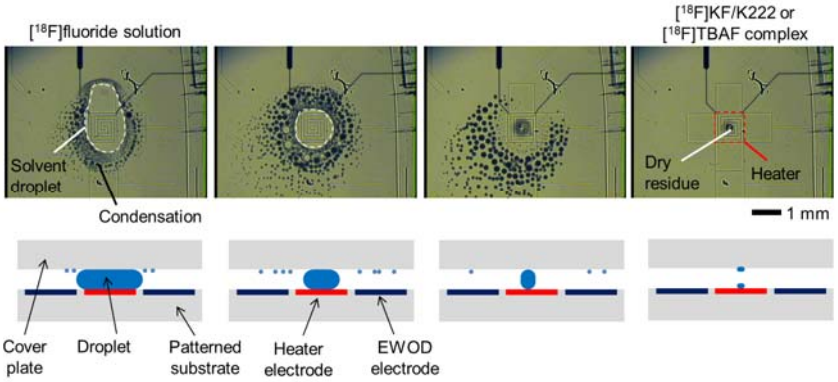

Figure 2: Evaporation process on EWOD chip. Top view micrographs (top row) and side view schematics (bottom row) of evaporation process to dry [^{18}F]fluoride on a previous EWOD chip design. Time progresses from left to right. When heated, the droplet evaporates from its open sides into vapor, which condenses on colder surfaces of the cover plate (immediately adjacent to heated area). Condensation increases as the droplet evaporates but slowly dissipates as the droplet disappears. Eventually, only the dried residue remains.

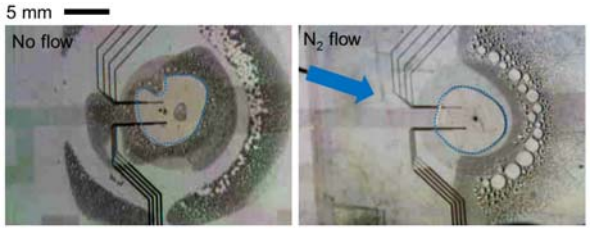

Figure 3: To facilitate removal of solvent vapor during evaporation steps, an inert gas is blown into the space between the parallel plates of the EWOD chip through a needle or vacuumed out through holes in a plate. (Left) Without gas flow, condensation occurs all around the heater site close to the evaporating droplet (blue outline) and can recondense in the droplet. (Right) With gas flow, the evaporated vapor is removed more effectively and evaporation is accelerated.

Unlike the majority of macroscale methodologies, we discovered for multi-step reactions on EWOD that it was unnecessary to completely remove the remaining DMSO after fluorination; the subsequent deprotection reaction could be efficiently performed immediately afterwards by simply adding the aqueous acid or base to the remaining droplet and heating. Analogous procedures can be followed for additional reaction steps, if applicable.

RESULTS AND DISCUSSION

Synthesis results are summarized in Table 1. Yields in most cases were found to be comparable or superior to reactions performed at the macroscale and exhibit low standard deviation, suggesting high reaction repeatability.

In some multi-step reactions ([^{18}F]FDG and [^{18}F]FLT), the remaining DMSO after the fluorination step does not seem to interfere with downstream reaction steps and time could be saved compared to the macroscale by performing hydrolysis without first evaporating the fluorination solvent.

CONCLUSION

Digital microfluidics is in many ways an ideal platform for numerous applications of batch chemical synthesis at the microscale. As a demonstration, we synthesized four radiotracers for PET on the EWOD chip, obtaining conversions that are comparable or superior to studies at the macroscale, and reduced synthesis times due to elimination of evaporation steps before hydrolysis.

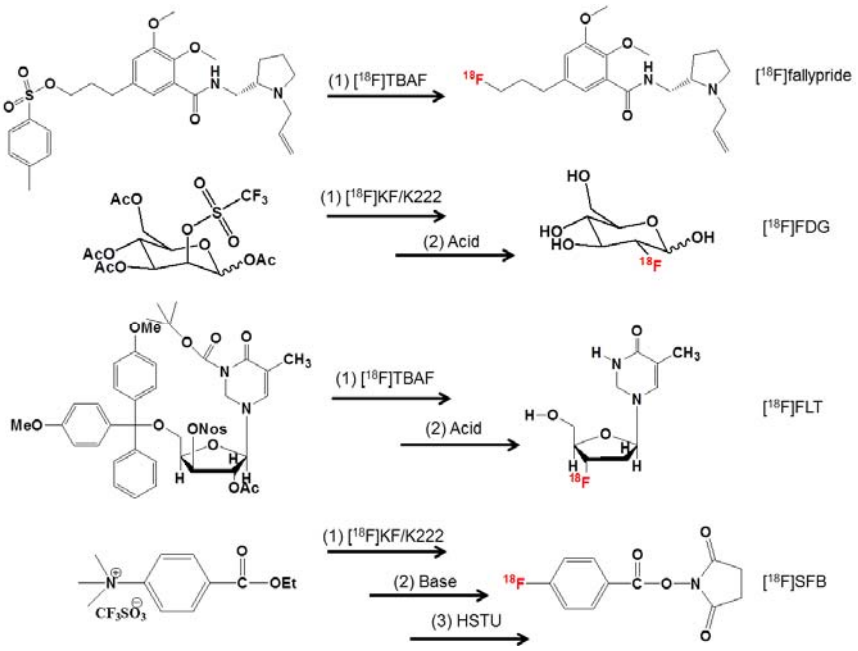

Figure 4: Synthesis schemes of diverse molecules prepared on the EWOD microfluidic chip: (a) [^{18}F]fallypride (1 reaction step), (b) [^{18}F]FDG (2 steps), (c) [^{18}F]FLT (2 steps), and (d) [^{18}F]SFB (3 steps). The indicated number of reaction steps takes place after the [^{18}F]fluoride complexation and drying process.

Table 1: Reaction yield for several single- and multi-step organic syntheses. Yield values for intermediate reactions are determined by radio-TLC as the fraction of radioactivity incorporated into the desired chemical intermediate from a tiny sample of the crude mixture. Yield values for final reaction are the conversion multiplied by the fraction of radioactivity that could be collected from the chip. Macroscale values are taken from recent literature.

Molecule	# of Reaction Steps	Decay-Corrected Yield (%)			Yield (%) (macroscale)
		Step 1	Step 1+2	Step 1+2+3	
[^{18}F]fallypride	1	72±10 (n=4)			20-40
[^{18}F]FDG	2	88±7 (n=11)	59±13 (n=5)		50-80
[^{18}F]FLT	2	78±6 (n=8)	50±8 (n=3)		10-40
[^{18}F]SFB	3	72±11 (n=4)	No data	39±6 (n=4)	25-75

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