SOLID-PHASE [^{18}F]FLOURINATION ON A FLOW-THROUGH GLASS MICROFLUIDIC CHIP

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ABSTRACT

Microfluidic radiosynthesizer and solid phase synthesis methodologies are attractive for rapid production of short-lived radiolabeled probes for positron emitting tomography (PET). Herein, we combined the synergistic properties of a microfluidic synthesizer embedded with a polymer supported precursor to enable diverse production of molecular probes at the point-of-use. In this first proof-of-concept, we have successfully demonstrated the solid phase radiosynthesis on a continuous flow glass microfluidic chip with 75% fluorination efficiency. The use of a simplified linker strategy on a polymerizable substrate within a microfluidic chip could be diversified to synthesize a wide range of PET probes.

KEYWORDS: Radiochemistry, F-18, solid phase synthesis, microfluidics, microchemistry

INTRODUCTION

Positron emitting tomography (PET) is a highly sensitive and non-invasive imaging technique for detecting biochemical processes *in vivo*.[1] Small molecule PET probes typically use fluorine-18 as the radioisotope of choice due to its relatively long half-life compared to other radioisotopes[2]. ^{18}F-labeling of small molecules is usually carried out by nucleophilic fluorination of precursors with no-carrier-added (n.c.a.) [^{18}F]fluoride ion to achieve high specific activity. The short half-life of fluorine-18 ($t_{1/2}$= 109 min) requires fast reaction rates and purification processes. Typically, radiofluorination reactions utilize huge excess of precursor relative to [^{18}F]fluoride ion to achieve >60% fluorination efficiency within 30 minutes of fluorination time. As a result, large amounts of unreacted precursors and side products are produced, which requires extensive purification processes. To streamline the purification processes, solid-phase radiosynthesis, in which excess precursor is supported onto a polymer matrix, has been reported for the synthesis of 2-[^{18}F]fluoro-2-deoxy-*D*-glucose in a macroscale reactor using the perfluoroalkyl sulfonate linker.[3,4] However, the synthesis of the sulfonate linker involved an expensive starting material and tedious preparation (four step synthesis), which limits the wide-spread use of this methodology for other PET probes.

Recently, microfluidic synthesizers have emerged for the syntheses of short-lived molecular probes due to their intrinsic properties, which leads to faster reaction kinetics, higher reaction selectivity, and allow for high degree of integration, reaction parallelism and automation.[5] Here, we combined the advantageous of both microfluidic radiosynthesizers and the polymer-supported radiosynthesis methodology for rapid fluorine-18 radiochemistry. To overcome the tedious and expensive preparation of the perfluoroalkylsulfonate ester reported earlier, we synthesized a simpler sulfonate ester linker functionalized with a polymerizable moiety starting from a commercially available building block in one-step. The reactive sulfonate monomer is directly polymerized within the microfluidic channels under visible light irradiation to yield a macroporous bed of polymer support, known as a polymer monolith[6]. The polymer monolith embedded with the substrate is then used as a model reaction towards the development of a rapid, simple and clean microscale solid phase radiosynthesis.

EXPERIMENTAL

As a proof-of-concept demonstration, we synthesized 3-phenylpropyl 4-vinylbenzenesulfonate from a commercially available 4-chlorosulfonyl styrene and 3-phenyl propan-1-ol. A mixture of 3-phenyl propan-1ol (0.136g, 1mmols) and triethylamine (1g, 10mmols) in 10 mL of methylene chloride was reacted with 2 equivalent of 4-chlorosulfonyl styrene (0.426, 2mmols) at 0˚C for 5 hours. The crude reaction was washed with 2M ammonium chloride and a brine solution to yield 3-phenylpropyl 4-vinylbenzenesulfonate in 62% (NMR yield). The off-the-shelf microfluidic chip channels (Micronit, dimension 150 µm(w) x 150 µm (d) x 52000 µm (l)) was first vinylized with 3:7 (v/v) ratio of methacryloxypropyltrimethoxysilane and acetone in order to anchor the porous monoliths onto the micro channels.[7] The purified vinyl benzene sulfonate ester monomer, (500mg, 3 mmol) and divinylbenzene (250 µL, 230 mg, 1.8 mmols) were dissolved in a mixture of porogens (acetonitrile (237 µL), isopropanol (500 µL) and 1-decanol (600 µL)) containing initiators, S-camphorquinone (3.2 mg, 0.18 mmols), ethyl-N-dimethylbenzoate (32 mg; 0.16 mmols), and N-methyl-pyridinium tetrafluoroborate (32 mg, 0.18 mmols). The polymerization mixture was flow into the vinylized glass microchip and was polymerized between two arrays of LED lights (Roithner Lasertechnik, 470 nm, 12x20mW) and irradiated for 15 min (Scheme 1).[8] . The resulting polymer monolith was washed with copious amount of THF (~ 5mL) to remove any residual starting materials using a syringe pump. The sulfonate ester polymer monolith was characterized via ATR-IR and solid phase ^{13}C NMR spectroscopies. Both IR and ^{13}C NMR spectroscopies confirmed the presence of the sulfonate ester linker on the polymer monoliths. (**Figure 1)**

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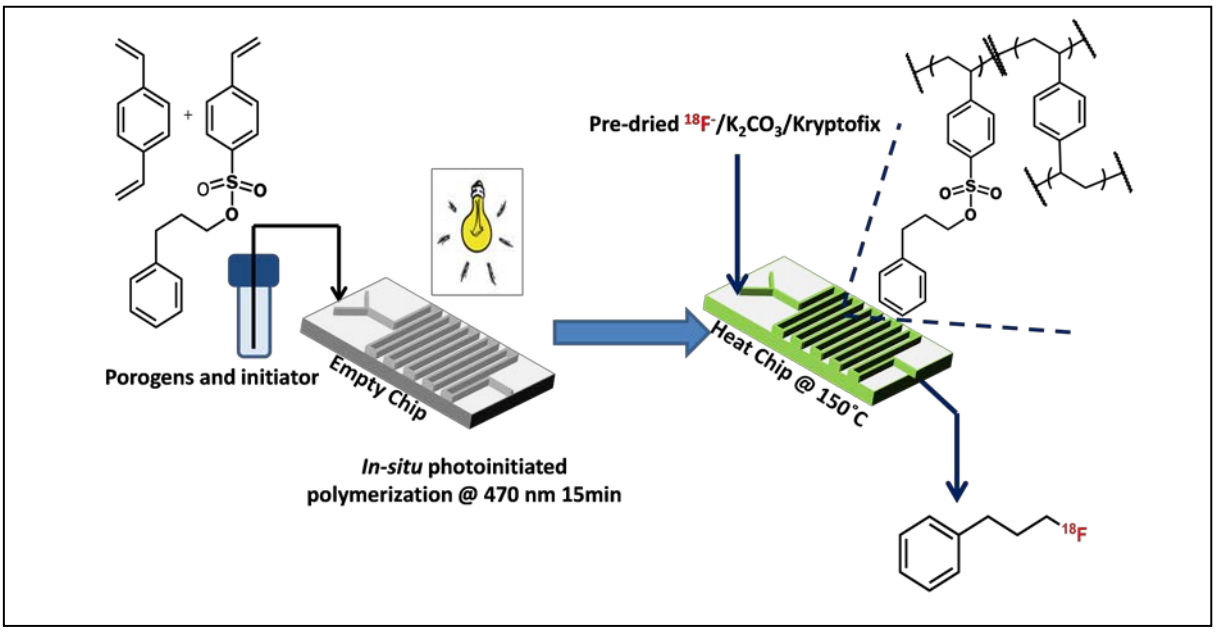

Scheme 1. Overall synthetic scheme of the polymer monolith with sulfonyl ester moiety via photo-initiated polymerization reaction in a flow-through glass microfluidic chip. A mixture of monomers, initiator and porogens was flowed into the microfluidic channels and irradiated for a predetermined time to form the macroporous sulfonate ester polymer monolith. The pre-activated K/[^{18}F]F/$K_{2.2.2}$ complex is flowed through the aryl sulfonate ester monolithic microchip and heated at 150 °C to perform the solid phase radiofluorination reaction. The activated [^{18}F]fluoride ion cleaves the sulfonate linker via nucleophilic substitution reaction and releases the fluorinated product into solution, while the unreacted precursor and side products remained on the polymer support.

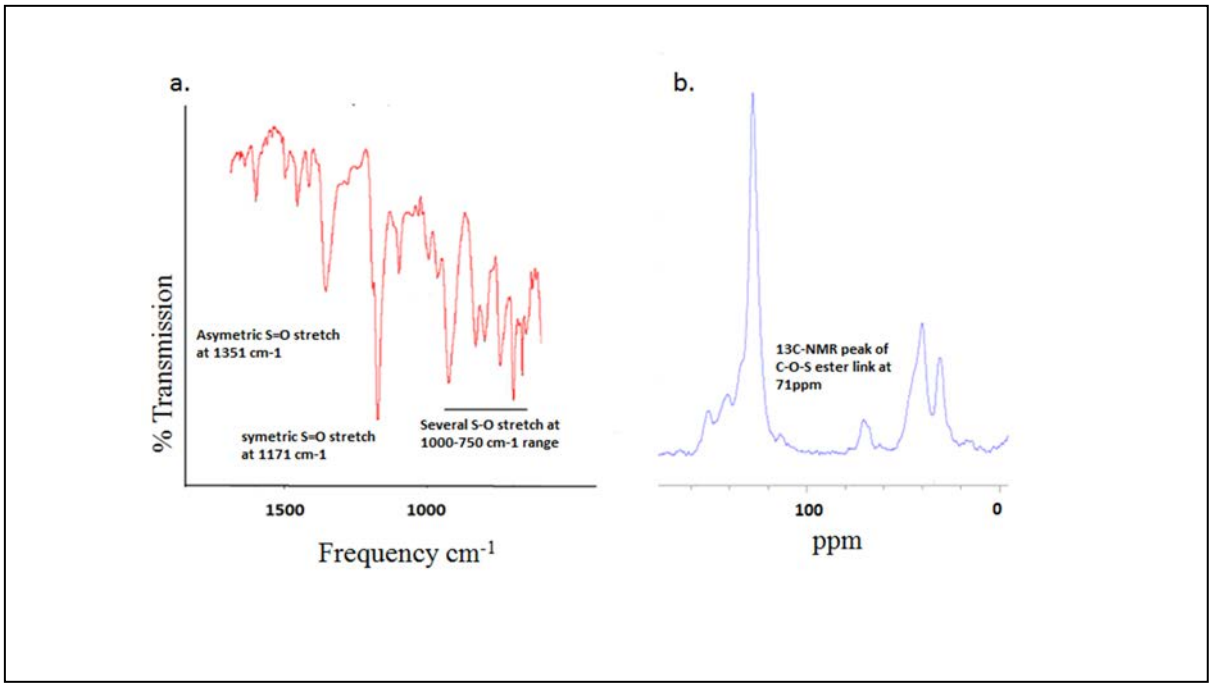

Figure1. Spectroscopic characterization of sulfonate polymer monolith (a) ATR-IR spectrum showing an asymmetric S=O stretch at 1351cm^{-1}, a S=O symmetric stretch at 1171 cm^{-1} and several S-O stretches at 1000-750cm^{-1} (b) Solid phase ^{13}C-NMR showed the presence of an ether bond (C-O), which confirms the formation of the sulfonate ester polymer monoliths.

The [^{18}F]fluoride/K_2CO_3/Kryptofix (K[^{18}F]F$^-$/$K_{2.2.2}$) complex was dried azeotropically to activate[^{18}F]fluoride ions on a Teflon-coated glass substrate as reported elsewhere.[9] The activated K[^{18}F]F$^-$/$K_{2.2.2}$ complex was transferred into a syringe using 100 µL of anhydrous acetonitrile and flowed through the embedded sulfonate ester monolith at a flow

rate of 1.5 µL/min (residence time of 6 min), while heating at 150˚C. The eluent was collected and analyzed via radio-TLC and radio-HPLC (Figure 2). The solid phase radiofluorination efficiency was found to be 75%, while the radio-HPLC confirmed the formation of ^{18}F-labeled product (retention time 9.3 min). This result confirmed that simple sulfonate ester is reactive towards nucleophilic radiofluorination when performed within a continuous flow microfluidic device. However, under this condition, other undesirable side products were also liberated along with the [^{18}F] fluorinated product as observed in the HPLC (UV traces) (Figure 2b). While this condition is yet to be optimized, the simplicity of the linker chemistry and the ability of the substrate to be directly polymerized *in-situ* within a microfluidic channels serves as a building block to achieve a rapid and clean radiosynthetic methodology.

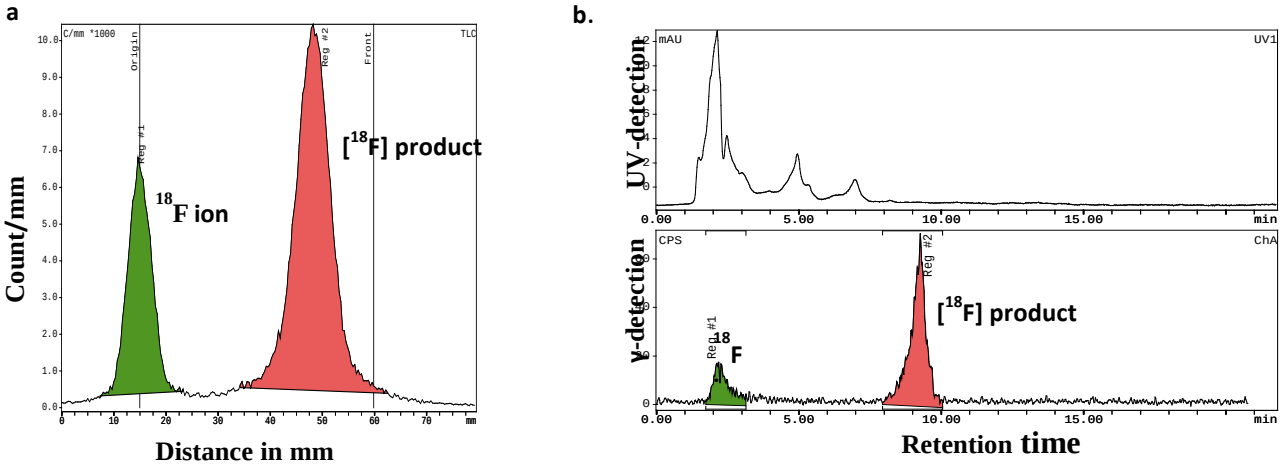

Figure 2. (a) Radio-thin layer chromatography developed in hexane/ethyl acetate (1:1 v/v) showing the radiolabeled (3-[^{18}F] fluoropropyl)benzene, which travelled at a distance of 50mm on the TLC plate while the unreacted [^{18}F]fluoride ion remained on the baseline. (b) HPLC analysis (UV and radio-detection) of the same crude sample, in which the ^{18}F-labeled product was eluted at 9.27 mins as observed in the radio-HPLC chromatogram.

CONCLUSIONS

In this report, we have synthesized the polymerizable 3-phenylpropyl 4-vinylbenzenesulfonate monomer followed by the in-situ copolymerization with divinylbenzene cross linker within a flow-through microfluidic chip. Upon optimization of the photopolymerization conditions, a macroporous bed of polymer supporting a simple precursor and a suitable linker for solid phase radiofluorination reaction was obtained. In conclusion we have demonstrated, for the first-time, solid phase radiosynthesis of a model substrate on a microfluidic device. The combination of microfluidics with solid phase synthetic methodology has the potential of enhancing the reaction kinetics, selectivity and the ability to automate the production of PET probes on demand.

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