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SuFEx-Based Strategies for the Preparation of Functional Particles and Cation Exchange Resins

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A predictable and reproducible number of sulfuric acid sites have been achieved for cation exchange resins by employing a mild SuFEx-based reagent system to effect the hydrolysis of fluorosulfonated polymer beads. The resultant poly(hydrogen sulfate) beads effectively demonstrate their utility as cation exchange resins in ion-capture experiments. Furthermore, their polyfluorosulfonated precursors have also proven to be suitable substrates for traditional SuFEx-type click reactions in both small molecule and protein immobilization applications.

Ion exchange resins are a versatile class of polymeric materials that have a number of important industrial and medical applications ranging from water treatment and compound purification to drug delivery and catalysis.[1-5] Among the most widely employed ion exchange resins are crosslinked styrene/divinylbenzene (DVB) copolymers bearing highly ionizable sulfonic acid moieties known as strong acid cation (SAC) exchange resins. SAC resins are typically prepared via the post-polymerization modification (PPM) of DVB-crosslinked polystyrene beads with excess concentrated sulfuric acid at elevated temperatures, normally over 100 °C,[6] resulting in the installation of the requisite sulfonic acid functionality for cationic exchange. While this protocol is an effective means for producing SAC exchange resins, it unfortunately generates an acidic, sulfate-rich waste stream that not only requires neutralization but also has the potential to form toxic byproducts that can pose additional environmental concerns.[7-9] This methodology also installs an unpredictable sulfonic acid density and substitution distribution, since the only way to manipulate these parameters is by varying sulfuric acid reaction time. A more benign synthetic approach to arrive at SAC resins involves the direct preparation of sulfonic acid containing crosslinked polystyrenes

from sulfonated monomers; however, given the poor solubility of the hydrophilic monomers in styrene-DVB mixtures, an alternative strategy that offers a controllable, environmentally-friendly method of SAC production could have a wide-ranging impact.

An interesting approach to address this issue has emerged from our recent efforts to expand upon the synthetic applications of the sulfur(VI) fluoride exchange (SuFEx) reaction developed by Sharpless and coworkers.[10] SuFEx chemistry harnesses the unique reactivity displayed by traditionally inert sulfonyl fluorides and fluorosulfonates toward *N*- and *O*-nucleophiles as well as silyl ethers in the presence of amidine or guanidine bases to arrive at the corresponding sulfonamide, sulfonate, or sulfate products (Scheme 1). This innovative "click" reaction technology has demonstrated its utility in numerous scientific disciplines including polymer synthesis, surface chemistry, drug discovery/medicinal chemistry, and protein functionalization.[11-16] Our most recent endeavor concerning SuFEx methodology focused on its application to the PPM of sulfonyl fluoride containing polymer brushes for the efficient functionalization of surfaces.[15, 17] During the course of these studies, it was discovered that when *tert*-butyldimethylsilyl (TBS)-protected benzyl or allyl alcohols were exposed to sulfonyl fluorides under standard SuFEx conditions, they did not afford the expected benzyl or allyl sulfonates, but rather were hydrolyzed to the corresponding sulfonic acids (Scheme S1).[17] This observation encouraged our further investigation into fluorosulfonate polymer hydrolysis as a means to arrive at poly(hydrogen sulfate) materials with the potential to act as SAC exchange resins. We therefore sought to prepare DVB-crosslinked polystyrene beads bearing fluorosulfonate moieties such as **2** and explore mild hydrolysis conditions to yield desired hydrogen sulfate variants of type **3** (Scheme 2). This approach is particularly advantageous as the proportion of sulfate groups present in the polymer backbone can be controlled by the initial selection of monomer ratios while avoiding the use of harshly acidic reaction conditions.

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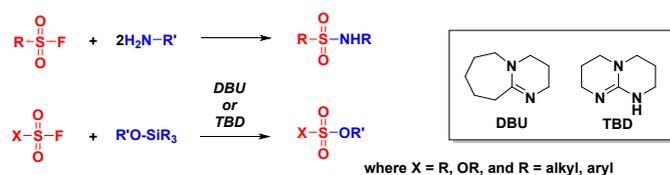
Scheme 1 General scheme for Sulfur(VI) Fluoride Exchange (SuFEx) Reaction

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In addition to ion exchange, polymeric microspheres also play an important role in many other applications, such as solid-support catalysis, liquid chromatography and enzyme immobilization.[18-20] The functional groups on the beads, whether introduced directly in the monomer or via PPM, are crucial to the applications mentioned above. In general, beads with low dispersity, versatile functionality, and a defined and reproducible amount of functional groups are of great interest. For these reasons, we also explored using the fluorosulfonated beads **2** as a polymer support to introduce several different functionalities via SuFEx click chemistry. Herein, we report an operationally simple approach to a novel class of SAC exchange resins, and also demonstrate a versatile platform for the fabrication of polymeric particles with utility in a variety of applications.
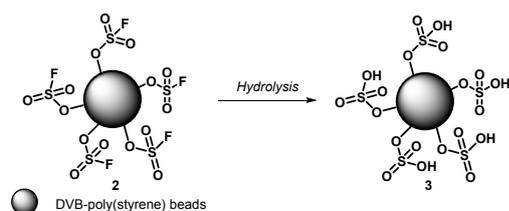
Scheme 2 Crosslinked polysulfuric acids via the hydrolysis of fluorosulfonated polymer beads

Our investigation into the feasibility of a SuFEx-based hydrolysis approach to SAC resins commenced with the synthesis of several DVB-crosslinked polymer substrates possessing varied degrees of fluorosulfonation. As illustrated in Scheme 3, the desired polymers were obtained via two different polymerization methods. Emulsion polymerization was first employed to generate polymers **6a-c** by slow treatment of a vigorously stirred aqueous solution of sodium dodecyl sulfate (SDS) and $(NH_4)_2S_2O_8$ with a mixture composed of

Beads	Composition
6a / 7a	VPSF + 10% DVB
6b / 7b	Sty/VPSF (70:30) + 10% DVB
6c / 7c	Sty/VPSF (90:10) + 10% DVB
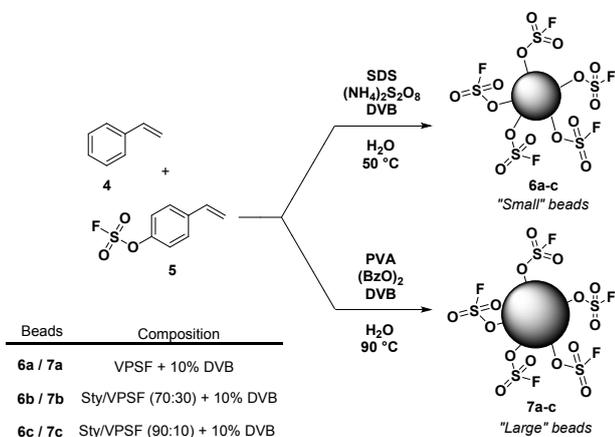
Scheme 3 Synthesis of DVB-crosslinked fluorosulfonated substrates via emulsion and suspension polymerization

10 wt% DVB and different ratios of styrene (**4**) and the previously described arylfluorosulfonate monomer, 4-vinylphenyl sulfofluoridate (VPSF, **5**).[17] Identical monomer ratios were then utilized for the suspension polymerization of styrene and VPSF whereby mixtures of **4** and **5** containing the radical initiator benzoyl peroxide and 10 wt% DVB were slowly added to an aqueous solution of 0.3% polyvinyl alcohol (PVA) to arrive at an additional three DVB-crosslinked polymers **7a-c**. While the composition of the respective polymers was the same, the choice of preparation method allowed for control over their particle size. Control of VPSF

loading into the bead was confirmed using elemental analysis (Table S2).

With sufficient quantities of the fluorosulfonated polymers **6a-c** and **7a-c** in hand, attention was then focused on their conversion to the corresponding polysulfuric acids. Application of our previously reported sulfonyl fluoride hydrolysis conditions involving benzyl silyl ethers and strong organic amine bases was anticipated to furnish the desired hydrolysis products **9a-c** and **10a-c** as outlined in Scheme 4.[17] Treatment of small and large beads **6a** and **7a** with TBS-benzyl alcohol **8** in the presence of substoichiometric amounts of triazabicyclocodecene (TBD) at 70 °C resulted in the near complete conversion to the desired sulfuric acid bearing polystyrenes as determined by IR spectroscopy. As can be seen from Figure S1, the strong absorption band at 1450 cm^{-1}, indicative of the asymmetric S=O stretching of fluorosulfonates, mostly disappeared after ~12 h at 70 °C while a prominent new peak appeared at 1045 cm^{-1} corresponding to the formation of a hydrogen sulfate product. FTIR spectra for the TBD-catalyzed reactions of polymer beads **6b/7b** and **6c/7c** also exhibited similar diagnostic absorptions at 1042 cm^{-1} indicating successful fluorosulfonate hydrolysis (Figs S2 and S3). Orthogonal confirmation of deprotection efficacy was obtained via elemental analysis for beads **7a-c** (Table S2) where TBD-catalyzed hydrolysis conditions resulted in near quantitative conversion of fluorosulfonates into hydrogen sulfate products in all cases.
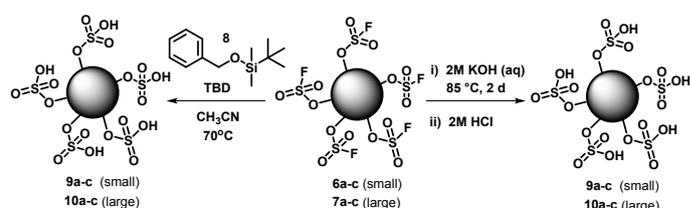
Scheme 4 TBD-catalyzed and base-mediated hydrolysis of fluorosulfonated polymer beads

Fluorosulfonate hydrolysis to the corresponding sulfuric acids was also speculated to occur under strongly basic aqueous conditions. To test this hypothesis, small and large polymer beads were suspended in 2 M KOH and maintained at 85 °C for 2 days (Scheme 4). The resulting solids were then characterized via FTIR and elemental analysis. In contrast to the TBD-catalyzed hydrolysis, polymers **6a-c** and **7a-c** demonstrated modest reactivity with FTIR revealing a mixture of flurorosulfonate starting material and hydrogen sulfate product (Figs S4 and S5). However, elemental analysis did not show significant conversion to the desired products (Table S2), potentially indicating surface hydrolysis of the polymeric resins under aqueous KOH conditions.

Further characterization of the polymer beads obtained from these basic hydrolysis experiments was achieved via zeta potential and dynamic light scattering (DLS). Zeta potential and DLS measurements obtained for the parent fluorosulfonate polymer beads **6a-c** and the corresponding hydrolyzed particles **9a-c** are presented in Table S1. Parent beads **6a-c** exhibited a negative zeta potential value of −30 mV most likely due to residual surface adsorbed SDS from the emulsion polymerization conditions. The remaining data for small beads **9a-c** shows zeta values ranging from −38 mV to −28 mV, respectively, which is consistent with the presence of highly ionizable sulfate moieties. The observed zeta potential variability correlates with the percentage of sulfate moiety incorporation as hydrolyzed polymer beads derived from precursors containing greater amounts of VPSF displayed a more

negative surface charge. Negligible change was observed in the case of beads **9c** which possessed the lowest percentage of VPSF monomer. Strong, negative zeta potentials also suggest moderately stable particles with a lower propensity for aggregation. Particle size determinations via DLS revealed a diameter of 120 nm for polymer beads **6a** containing only DVB-crosslinked VPSF **5** while polymers **6b** and **6c** containing 30% and 10% VSPF, respectively, were significantly smaller measuring 67 and 80 nm in diameter. Hydrolyzed variants **9a-c**, as expected, displayed larger diameters than the parent. Due to difficulty in identifying a suitable solvent to suspend large beads **7a-c**, no zeta potential or DLS measurements were obtained for these examples.

The morphology of large fluorosulfonate beads **7a-c** under different treatments was studied using SEM (Figs. 1 and S6). Parent fluorosulfonate polymer beads were all spherical particles ranging from 10 to 100 μm in diameter. However, subtle differences were observed at the outer surface of the particles, where large beads **7a** showed several surface defects and **7b** had a "wrinkling skin" that likely originates from the immiscibility of the monomers and oligomers during the polymerization process (Fig. 1A-C). Both beads **7b** and **7c** are composed of styrene and VPSF monomers, however, the majority of large beads **7c** are much smoother due to their relatively low percentage of VPSF. In comparing the two hydrolysis conditions, the KOH treatment yielded damaged particles with significant etching that either presented a hairy microstructure (Fig. 1D) or cracked holes (Fig. 1E-F). Treatment with TBD and **8** is much milder, and maintains the intact particles with only a few surface defects or cracks (Fig. 1G-I). This can be explained by the basicity of the solution and the agitation and heating time. Aqueous 2M KOH solution is more basic than the millimolar TBD reagent, resulting in more etching. The milder TBD conditions not only resulted in more intact spherical resins, but also afforded a superior amount of sulfate groups based on FTIR and elemental analysis.

In order to evaluate the ability of these hydrolyzed polymeric products to serve as SAC exchange resins, two additional studies were conducted with large beads **10a-c**. Initially, a simple ion capture experiment was performed with the synthetic cationic dye, methylene blue. Polymer beads **7a-c** and the corresponding hydrolyzed particles **10a-c**, prepared via both KOH-mediated and TBD-catalyzed routes, were treated with a 1 mg/mL solution of methylene blue in deionized water. As illustrated in Fig. 2, the parent compounds **7a-c** did not retain any blue coloring while the images of hydrolyzed products **10a-c** indicate the successful capture of the cationic blue dye. The amount of color retained by the new cation exchange resins qualitatively correlates to the percentage of sulfate moieties present on the polymer beads as the beads containing greater amounts of VPSF displayed a darker blue color. Moreover, resins obtained via TBD-catalyzed hydrolysis (Fig. 2) exhibited a darker coloring compared to their base-mediated hydrolysis counterparts (Fig. S7B) indicative of the more complete level of hydrolysis achieved with TBD-catalysis over aqueous KOH conditions.

Next, the ion exchange capacity (IEC) of hydrolyzed beads **10a**-**c** was investigated to gain a quantitative measure of sulfuric acid functionalization. IECs were determined via a standard titration protocol[6] and compared to theoretically established values (Table S3). Resins **10a-c** were found to exhibit IEC values of 2.7, 1.2, and 0.45 mequiv g^{-1}, respectively. As predicted, resins derived from polymers with lower amounts of VPSF, displayed correspondingly lower IECs. These results show a reasonable correlation between the theoretically calculated IEC values as well as literature values for similar sulfonated resins.[6] It is also confirmed that resin IEC can be

directly controlled by the initial monomer ratio allowing for the predictable and reproducible incorporation of acidic functionality. Overall, these preliminary results effectively demonstrate the utility of poly(hydrogen sulfate) beads as cation exchange resins.
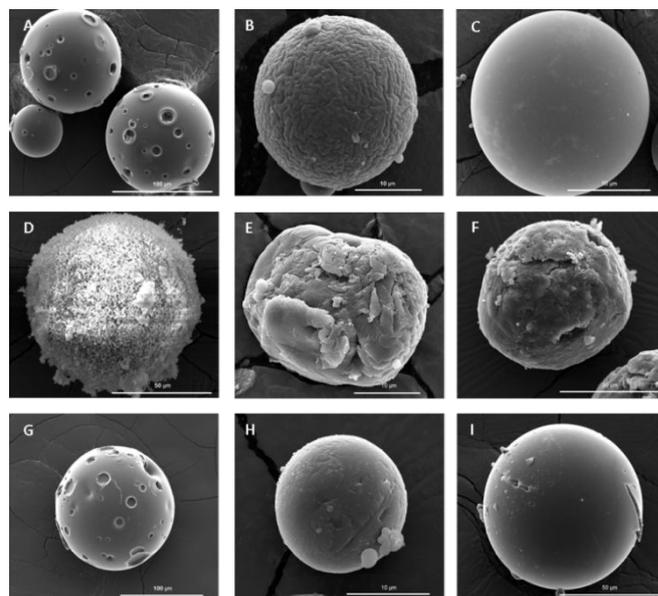
Fig. 1 Scanning electron micrographs of original and deprotected large fluorosulfonated beads. (A-C) Large fluorosulfonated beads **7a-c** before deprotection, (D-F) deprotected beads from aqueous base hydrolysis, and (G-I) deprotected beads via TBD-catalyzed hydrolysis.

In addition to demonstrating their ability to undergo hydrolysis to arrive at SAC exchange resins, polyfluorosulfonate precursors **6** and **7** have also proven to be suitable substrates for traditional SuFEx-type click reactions. Both large and small beads with different compositions obtained from suspension and emulsion polymerization, respectively, were treated with a simple silyl-protected reacting partner, TBS-phenol **11**, in the presence of 20 mol% DBU at 60 °C (Scheme 5). Under these standard SuFEx reaction conditions, the desired phenyl sulfate product **12** was formed as detected by IR spectroscopy (Figs. S8-10). All beads in different compositions resulted in the appearance of two new peaks, ~1415 and ~1216 cm^{-1}, that originate from the formed phenyl sulfate groups. Polymer beads containing a lower amount of VPSF, such as beads **6c** and **7c**, showed weaker signals in FTIR spectra (Fig. S10) due to fewer conjugation sites.
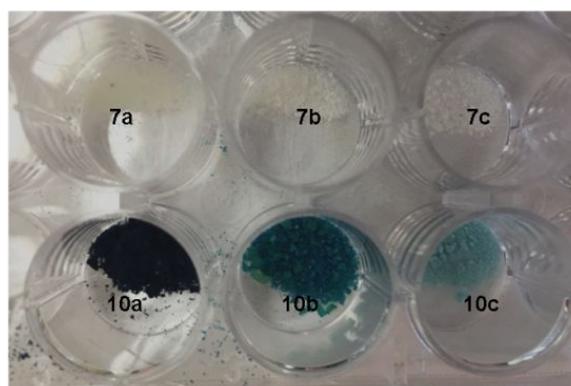
Fig. 2 Methylene blue capture experiment. Parent and hydrolyzed DVB-crosslinked polymer beads obtained via TBD-catalyzed hydrolysis following aqueous methylene blue treatment.

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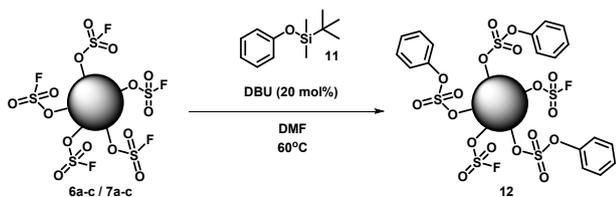
Scheme 5 TBS-phenol SuFEx click on fluorosulfonated polymer beads

Given the observed compatibility with SuFEx reaction conditions, we attempted to further extend the scope of our DVB-crosslinked fluorosulfonated beads by utilizing them as a support for the immobilization of enzymes. Enzyme immobilization (EI) has become a popular strategy to more effectively harness the excellent selectivity and specificity exhibited by enzymes for industrial applications.[21-24] By enabling enzyme recycling, enhancing stabilization, and allowing for easy separation from reaction mixtures, EI presents distinct advantages over the use of soluble enzymes for biocatalytic transformations.[25, 26] Recently, we demonstrated the utility of SuFEx methodology for protein-polymer bioconjugation through the chemoselective functionalization of the commercially available enzyme, bovine serum albumin (BSA).[16] For the present study, we sought to evaluate the potential of our new fluorosulfonated polymers to immobilize lipase from the thermophilic fungus, *Thermomyces lanuginosus* (TL), and subsequently test the activity of the resultant conjugate.
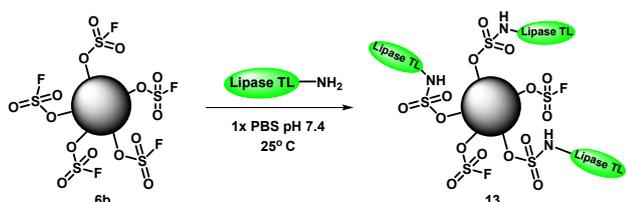
Scheme 6 Immobilization of Lipase TL on fluorosulfonated polymer beads

The previously described SuFEx ligation chemistry[16] was employed to modify the DVB-crosslinked styrene/VPSF (7:3) beads **6b** with lipase TL and also with green fluorescent protein (GFP) as a control. The reaction of DVB-crosslinked styrene/VPSF beads with lipase TL in aqueous buffer solution furnished the desired protein-polymer conjugate **13** (Scheme 6). Lipolytic activity of **13** (sty/VPSF-TL) was determined spectrophotometrically by using *p*-nitrophenyl palmitate (*p*-NPP) as substrate and compared to both lipase in solution (TL) and immobilized GFP (sty/VPSF-GFP). While lipase conjugate **13** exhibited lower activity than the native lipase TL (Fig. S11), further optimization of the immobilization conditions and enzyme loading could be investigated to enhance enzymatic activity and will be the subject of a future study.

In summary, we have discovered an operationally simple and controllable strategy for the fabrication of SAC exchange resins. Mild SuFEx-based hydrolysis conditions allowed for the installation of a predictable and reproducible number of sulfuric acid sites while maintaining overall bead structure and morphology. The resulting hydrolyzed beads effectively demonstrated their ion-exchange capability in dye capture experiments and IEC determinations. In addition, precursor DVB-crosslinked polystyrene beads bearing fluorosulfonate moieties were shown to undergo conventional SuFEx chemistry using aromatic silyl ethers and amino conjugation with lipase. This methodology thus offers a feasible and versatile approach to fabricate multifunctional polymer-supported beads with utility across various applications of chemistry and biology.

Conflicts of interest

There are no conflicts to declare.

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