PEO-based brush-type amphiphilic macro-RAFT agents and their assembled polyHIPE monolithic structures for applications in separation science

Aminreza Khodabandeh1,2, R. Dario Arrua2, Fotouh R. Mansour1,3, Stuart C. Thickett4 & Emily F. Hilder2

Polymerized High Internal Phase Emulsions (PolyHIPEs) were prepared using emulsion-templating, stabilized by an amphiphilic diblock copolymer prepared by reversible addition fragmentation chain transfer (RAFT) polymerization. The diblock copolymer consisted of a hydrophilic poly(ethylene glycol) methyl ether acrylate (PEO MA, average Mn 480) segment and a hydrophobic styrene segment, with a trithiocarbonate end-group. These diblock copolymers were the sole emulsifiers used in stabilizing “inverse” (oil-in-water) high internal phase emulsion templates, which upon polymerization resulted in a polyHIPE exhibiting a highly interconnected monolithic structure. The polyHIPEs were characterized by FTIR spectroscopy, BET surface area measurements, SEM, SEM-EDX, and TGA. These materials were subsequently investigated as stationary phase for high-performance liquid chromatography (HPLC) via in situ polymerization in a capillary format as a ‘column housing’. Initial separation assessments in reversed-phase (RP) and hydrophilic interaction liquid chromatographic (HILIC) modes have shown that these polyHIPEs are decorated with different microenvironments amongst the voids or domains of the monolithic structure. Chromatographic results suggested the existence of RP/HILIC mixed mode with promising performance for the separation of small molecules.

Macroporous polymer materials with interconnected structures represent a useful class of polymers used in different fields including separation science in the last decades1. An increasingly exploited method for the preparation of highly porous scaffolds is based on the solidification of the continuous phase of a high internal phase emulsion (HIPE) through polymerization. A cellular monolithic structure, commonly with interconnected pores and hence an open cellular network is produced, referred to as a poly(HIPE)2–7. These materials have been applied extensively to different applications including membrane separator for batteries9–12, electro-chemical sensors13, tissue engineering14–17, supported catalysis18, water purification19,20, and separation science21–24. All the demonstrated examples in separation science consist of polymers that are hydrophobic in nature, which limits their applications to separation of non-polar analytes in reversed-phase mode25. Introducing polar functional groups in the developed poly(HIPE) makes possible the separation of such analytes of different polarities.

PolyHIPEs with a hydrophilic surface are able to be produced through several different methods: post-synthesis modification of hydrophobic polyHIPEs from water-in-oil (w/o) HIPEs26–28, the synthesis of inverse HIPEs (using an oil-in-water (o/w) template) in which the monomer is placed in aqueous phase29–32, or the synthesis of bi-continuous hydrophobic polyHIPEs wherein a hydrophilic co-monomer is placed in the aqueous phase of an internal phase in w/o HIPEs33–36. Viswanathan et al. developed a new method for direct hydrophilic functionalization of a hydrophobic polyHIPE by introducing commercially available polymeric surfactants

1Australian Centre for Research on Separation Science (ACROSS), University of Tasmania, Tasmania, Australia. 2Future Industries Institute, University of South Australia, Building X, Mawson Lakes Campus, GPO Box 2471, Adelaide, SA 5001, Australia. 3Department of Pharmaceutical Analytical Chemistry, Tanta University, Tanta, Egypt. 4School of Physical Sciences, University of Tasmania, Private Bag 75, Hobart, 7001, Australia. Correspondence and requests for materials should be addressed to E.F.H. (email: emily.hilder@unisa.edu.au)
into a w/o HIPE through physical or chemical entanglement. 3D surface functionalization was obtained in which the hydrophilic part of the polymeric surfactant (such as acrylic acid groups) decorated the surface of the voids of the obtained polyHIPE. Mathieu et al. reported the synthesis of a hydrophilic surface modified polyHIPE using an amphiphilic macro-RAFT agent for stabilization of the HIPE template. The presence of RAFT functionality at the chain end of the polymer in the oil phase (styrene and divinylbenzene) provides a possibility for preparation of the porous polymer under RAFT control.

In our previous study, an amphiphilic copolymer (a “macro-RAFT agent”) was used as an anionic emulsifier in an inverse HIPE approach. This method offers attractive possibilities for the development of special coatings of the resultant hydrophilic polyHIPE after the curing step while the RAFT-end group remained at the surface. Our aim is to develop a surfactant-assisted functionalization strategy for preparation of porous polymers by HIPE polymerization, whereby the obtained porous polymers have a specific application.

The preparation of a hydrophilic polyHIPE from an o/w HIPE usually requires more careful emulsion stabilization than normal HIPE (w/o HIPE). The use of PEO-based “brush-like” monomers is anticipated to increase stabilization due to a larger surface area occupied per chain and the higher surface mobility of PEO chains. PEO has been found to provide surfaces with anti-fouling properties as a result of its hydrophilicity, high surface water mobility and low interfacial free energy with water. PEO-based macromolecules have demonstrated their unique potential as steric stabilizers for emulsion polymerization and may enhance their stability against freeze-thaw or shear force. We hypothesize that the PEO-based brush-type amphiphilic macro-RAFT agents with appropriate wettability will be adsorbed at the toluene–water interface, in a similar fashion as polymeric surfactant, and will provide stability against coalescence of the oil droplets, while the PEO block anchoring assists the attachment of these polymeric surfactants to the surface of the obtained polyHIPE upon polymerization (Fig. 1).

As these polymers adopt the format of the mold used as the reactor, an inverse high internal phase emulsion can be introduced into capillary tubing and by in situ polymerization of the continuous phase, it can be covalently attached to the surface modified silica capillary. Due to the aspect ratio of the capillary, the morphology of the hydrophilic polyHIPE is likely to differ to that of the bulk material, representing a synthetic challenge to replicate ideal conditions to prepare a porous monolithic structure. These monolithic columns can potentially offer several advantages in the design of high performance columns to be used in liquid chromatography including the high porosity and consequently a low resistance to the mass transfer (low C-term in the van Deemter equation). In addition, the active chain end (the RAFT-end group) sits at the surface of the material, and its role can be readily studied with respect to potential further surface functionalization.

In this work, the surface chemistry of a hydrophilic polyHIPE inside a capillary format was studied by liquid chromatography. This technique was particularly informative, revealing the role and relevance of the surface chemistry of the polyHIPE with respect to the retention time of different compounds in different modes of chromatography.

Results and Discussion

Typical synthesis of the amphiphilic quasi-block macro-RAFT agent. The increasing importance and interest in macro-RAFT block copolymers arise mainly from their unique amphiphilic properties in solution, which are a direct consequence of their molecular structure and presence of the RAFT-end group. While surfactants are selected mostly on trial and error basis for preparation of HIPEs, the hydrophilic-lipophilic balance (HLB) can indicate the capability of forming a certain preferred type of an emulsion. In case of HIPEs, surfactants with the HLB values 2–6 are used for water-in-oil and 12–16 for oil-in-water HIPEs. Specifically targeting a high HLB number (HLB ~16), amphiphilic macro-RAFT agents were synthesized to investigate the effect of the length of the P(PEO MA) and P(Sty) of the macro-RAFT agent with regards to the stability of the inverse HIPE. Table 1 shows the characteristic data for the P(PEO MA)-qb-P(Sty) diblock copolymers synthesized in this study. The SEC analyses of four different macro-RAFT agents are illustrated in Figure S1.
This one-pot polymerization technique has been utilized to achieve the synthesis of quasi (block-like) copolymers using sequential monomer addition. This approach yields quasi-block copolymers (Qb) when the conversion of monomer in the first step (e.g. PEO MA) is lower than 100% prior to a second monomer being incorporated. The low dispersity (Đ) of macro-RAFT agent Qb-1 to Qb-3 highlights the RAFT control over the polymerization. These results confirmed that shorter chain length of PEO MA macro-RAFT agents provide high reinitiation efficiency for the polymerization of Sty, as expected based on a previous report.

**Stability of oil-in-water HIPEs using PEO-based macro-RAFT agent.** The poly(PEO MA-qb-Sty) quasi-block copolymers prepared here are amphiphilic and can exhibit properties similar to a polymeric surfactant. As a starting point, macro-RAFT agent Qb-2 was chosen (Table 2). The use of 10 wt% of macro-RAFT agent Qb-2 resulted in the successful stabilization of HIPEs with aqueous volume fractions between 60 and 90%. The emulsion droplets were spherical but polydisperse (See Fig. 2 and Figure S2). The drop test method indicated that the HIPE was the inverse system (o/w type) (see Supporting Information Figure S3).

Preparation of a stable HIPE requires rapid adsorption of the stabilizer at the oil-water interface to lower the interfacial tension between the phases and form a rigid interfacial film. This process yields quasi-block copolymers (Qb) when the conversion of monomer in the first step (e.g. PEO MA) is lower than 100% prior to a second monomer being incorporated. The low dispersity (Đ) of macro-RAFT agent Qb-1 to Qb-3 highlights the RAFT control over the polymerization. These results confirmed that shorter chain length of PEO MA macro-RAFT agents provide high reinitiation efficiency for the polymerization of Sty, as expected based on a previous report.

| Sample code | macro-RAFT agent | wt% | Monomers in aqueous phase | HIPE stability (hours) |
|-------------|------------------|-----|--------------------------|------------------------|
| A1          | Qb-1             | 10  | —                        | >12                    |
| A2          | Qb-2             | 10  | —                        | >15 days              |
| A3          | Qb-3             | 10  | —                        | >12                    |
| A4          | Qb-4             | 10  | —                        | >1                    |
| A5          | Qb-2             | 10  | AAM-MBAM                 | >72                    |
| A6          | Qb-2             | 20  | AAM-MBAM                 | >72                    |
| A7          | Qb-2             | 50  | AAM-MBAM                 | >72                    |
| A8          | Qb-2             | 10  | AAM-MBAM - 20 wt% more monomers* | >24                    |
| B1          | RAFT-end group removed Qb-2 | 10 | AAM-MBAM - 20 wt% more monomers* | >24 |

**Table 2.** Conditions used for the preparation of inverse HIPEs. All amounts are based on the weight percentage (w.r.t. the continuous phase). The surface of capillary was modified with 3-(trimethoxysilyl)propyl methacrylate (γ-MAPS). This HIPE was stable for more than 15 days after preparation. Monomers in aqueous phase are acrylamide (AAM) and N,N'-methylenebisacrylamide (MBAM). The amount of the monomers is increased by 20 wt% respect to HIPE A5. For information of voids and windows size see the Table S2.

This one-pot polymerization technique has been utilized to achieve the synthesis of quasi (block-like) copolymers using sequential monomer addition. This approach yields quasi-block copolymers (Qb) when the conversion of monomer in the first step (e.g. PEO MA) is lower than 100% prior to a second monomer being incorporated. The low dispersity (Đ) of macro-RAFT agent Qb-1 to Qb-3 highlights the RAFT control over the polymerization. These results confirmed that shorter chain length of PEO MA macro-RAFT agents provide high reinitiation efficiency for the polymerization of Sty, as expected based on a previous report.

**Synthesis of hydrophilic polyHIPEs.** A rapid curing of a HIPE system typically locks the emulsion against Ostwald ripening and coalescence, resulting in a homogeneous polyHIPE structure. Inverse HIPEs discussed in the previous section were polymerized using a redox initiation system “potassium persulfate (KPS) /N,N,N',N'- tetramethylenehexanediamine (TEMED)” to obtain porous polyHIPEs. PolyHIPE A5 was obtained by using 10 wt% of macro-RAFT agent Qb-2, which retained the shape and volume of the mold. Increasing the
Macro-RAFT agent-Qb2 concentration from 10 wt% to 50 wt% had a significant effect on the morphology of the resulting polyHIPEs (e.g. on the void size ref. 55) as can be seen from the scanning electron microscopy (SEM) images (Fig. 3). From the SEM images, the number of “windows” per void is increasing from polyHIPE A5 to A7. A higher degree of openness is an advantage for polyHIPEs used in flow-through applications, as it decreases the backpressure of the column once the polyHIPE is introduced to a column housing.

The prepared polyHIPEs retained their yellow color after washing process due to the trithiocarbonate group of the RAFT agent. Elemental analysis confirmed the amount of sulfur within the polyHIPEs (e.g. the sulfur content within polyHIPE A5 was 0.43%). Further evidence for the presence of the macro-RAFT agent on the surface of the polyHIPE was obtained from Energy Dispersive X-ray analysis (EDX), clearly indicating that sulfur was present at the surface of the polyHIPE A5 (Fig. 4).

To further investigate the inclusion of the macro-RAFT agent within the polyHIPE structure, Fourier transform infrared spectroscopy (FTIR) analyses were performed on the resultant material, in comparison to a sample of AAM-MBAM polymerized in bulk (KPS/TEMED as initiators) subjected to the same washing protocol. The FTIR spectrum of polyHIPE A5 shows the presence of an extra band at 1710 cm\(^{-1}\) with respect to bulk polymer, which is present in the FTIR spectrum of the macro-RAFT agent (Fig. 5). This signal corresponds to the C=O stretching of the ester group of the PEO MA block, indicating incorporation of the macro-RAFT agent in to the
polymer structure. Furthermore, TGA thermograms of the macro-RAFT agent-Qb2, polyHIPE A5 and bulk polymer (see Figure S5 in the Supplementary Information) show similarities in the decomposition profile of polyHIPE A5 and Qb-2, again indicating macro-RAFT incorporation.

Grafting experiments utilizing the RAFT-end groups at the polyHIPE surface were performed aiming to demonstrate the presence of the reactive RAFT agent on the surface of voids. Adapting a procedure from Barlow et al.56, polyHIPE A5 was reacted at 60 °C overnight with (4-vinylphenyl) boronic acid (VPBA). Accordingly, a polyHIPE A5 was treated with a degassed solution of VPBA, RAFT agent and the initiator AIBN (molar ratios 100:5:1) in methanol–acetonitrile (volume ratio 50: 50) at 60 °C for 22 hours. FTIR spectroscopy was used to confirm the presence of poly(VPBA) on the surface, via the presence of B–O stretching peaks (see Figure S6, Supplementary Information)57, 58. SEM analysis (Fig. 6) demonstrated a change in surface morphology after surface grafting with VPBA where the size of the windows were decreased. These results clearly demonstrate the availability of the trithiocarbonate group (present on the surface of the functionalized polyHIPEs) for further surface modifications by grafting reactions.

In situ synthesis of hydrophilic polyHIPEs inside a capillary format. A capillary column was chosen as the reactor for the design of hydrophilic polyHIPEs to be used as a stationary phase in chromatographic experiments. The surface of the column was chemically modified with γ-MAPS in order to ensure a covalent
attachment between the polymer monolith and the walls of the capillary, subsequently ensuring the mobile phase would flow solely through the voids of the monolith. HIPE A5 was introduced to three different ID capillaries (150, 250, and 500 μm). After preparation of the HIPE A5, the HIPE was placed in ice bath for approximately 5 minutes to lower the temperature of the emulsion. As polymerization commences upon addition of TEMED, this stage is critical with respect to retarding the polymerization of the HIPE, providing sufficient time to fill the capillary using nitrogen gas. After the pre-treated capillary was completely filled with the cold HIPE, the capillary was sealed at both ends with rubber stoppers. The sealed capillary was stored in a dark place at room temperature and allowed to react for 24 h.

As seen in Fig. 7, the morphology of the resulting polyHIPE is strongly dependent on the size of the capillary. The morphology of the polyHIPE in the 500 μm ID capillary is most similar to the bulk structure (see Fig. 3, polyHIPE A5), however there is no attachment to the capillary surface. By decreasing the size of the capillary to 150 μm ID, the polyHIPE structure is attached to the surface but the morphology of the polyHIPE changes significantly. This result may be attributed to the deformability of oil droplets when the column is filling under nitrogen pressure, by considering the increasing likelihood of deformation and or break-up of when the inner diameter is decreased. Furthermore, the extent of polyHIPE shrinkage upon polymerization was studied (see Figure S7 in the Supplementary Information).

The shrinkage of the polyHIPE structure is ~19% (18.95 ± 4.20%). This is an important factor respect to explaining the de-attachment of the polyHIPE to wall in a larger inner diameter capillary. When the amount of the monomer-crosslinker (AAM-MBAM) in the aqueous phase was increased by 20 wt% (sample A8, Table 2), SEM analysis of the resulting polymer (sample A8, Fig. 8) showed a polyHIPE structure within a capillary housing similar to the bulk morphology.

Evaluation of the effects of RAFT-end group of the macro-RAFT agent on polyHIPE morphology. We next turned our attention to the role of RAFT-end group of the macro-RAFT agent. It has been reported that the presence of the RAFT-end group in amphiphilic copolymers increases the hydrophobicity of the copolymer46. This influences the behavior of a diblock copolymer at an oil-water interface, as it more closely resembles and acts as triblock copolymer.

To investigate this, the RAFT part of the macro-RAFT agent Qb-2 (See Table 1) was cleaved using a typical end group removing protocol with minor modifications (see Supplementary Information)59. Using this copolymer as a sole stabilizer, a stable inverse HIPE (toluene in water) was obtained. The stability of the HIPE stabilizing by end group removed Qb-2 was investigated by optical microscopy. It was found that both the toluene droplet size and the morphology of the obtained polyHIPE changed. An SEM image of the obtained polyHIPE is shown in Fig. 9. In comparison to polyHIPE A8 (Fig. 8), Fig. 9 shows that polyHIPE B1 possess a hierarchical polyHIPE structure with an increased number of windows. This increased level of interconnectivity was demonstrated with three-fold increase in BET specific surface area (6.75 m²g⁻¹ for B2, as opposed to 2.07 m²g⁻¹ for B1).

Our experience with hydrophilic polyHIPEs produced via inverse HIPEs stabilized by Tween 85 (a commercially available, non-ionic surfactant) with paraffin oil as the dispersed phase showed that there is no attachment of this polyHIPE to the modified walls of a capillary format column. Similarly, no attachment of the polyHIPE B1 to the surface of the column was observed in a 150 μm ID capillary (see Figure S8 in the supplementary information). The procedure was repeated in triplicate. The main difference between HIPE B1 and A8 is the presence (or not) of the trithiocarbonate group in the stabilizer used. These results suggest that an end-group removed RAFT copolymer will favor the formation of a hierarchically structured polyHIPE with no attachment to the capillary format column, in comparison to the copolymer cotaining RAFT-end group which enabled full attachment of the polyHIPE to the capillary wall. We believe that the RAFT-end group of the macro-RAFT agent group assures that the monolith is covalently adhered to the capillary (as the polyHIPE is remained attached to the wall after washing with a high pressure), guaranteeing the flow of liquid through the synthesized monolith. Upon removing the butyltrithiocarbonate endgroup, the anchor is changed in the way that the attachment to the capillary wall is not provided.
Evaluation of hydrophilic polyHIPE as stationary phases in HPLC. These as-prepared polymer monoliths in a capillary housing were then evaluated as stationary phases for nano-liquid chromatography. Interactions between analytes (with different polarity) and the polyHIPEs could give us information about the
different microenvironments present on the polymer surface. Two capillary columns containing polyHIPE A5 or A8 were studied. The suitability of the polyHIPE structure monoliths was assessed by measuring the backpressure of the materials at different flow rates. The backpressures obtained when both non-swelling (acetonitrile) and swelling (MiliQ-water) solvents were pumped through the polymeric monolith A8 shown in Figure S9. Due to the poor mechanical stability of the polyHIPE A5, it is unsuitable as a stationary phase and this sample was not investigated further (Fig. 10).

Considering the presence of amphiphilic copolymers on the surface of polyHIPEs, the materials are expected to allow the separation of both polar and non-polar analytes. The styrene part in the prepared polyHIPE induces hydrophobic interactions with nonpolar analytes while the surface coverage with PEO MA helps to retain polar

**Figure 9.** Scanning electron micrographs of emulsion templated macroporous polymer (B1) made by polymerization of HIPEs stabilized solely by end group-removed macro-RAFT agent-Qb2, polymerized at room temperature (KPS/TEMED).

**Figure 10.** After washing of polyHIPE with water using nano-LC HPLC system.
Analytes. The mechanism of chromatographic retention was studied using two different classes of compounds: non-polar alkylbenzenes to test for the reversed-phase (RP) mode and polar hydroxybenzoic acids to test for the aqueous normal-phase in hydrophilic interaction liquid chromatography (HILIC). Upon injecting the alkylbenzene mixture, the following elution order was observed:

Tolene < ethylbenzene < propylbenzene < butylbenzene < pentylbenzene

Although this order is typical for reversed-phase mode, the relationship between the length of the aliphatic chain (nc) and the logarithm of the retention factor was nonlinear as shown in Fig. 11-A. This can be explained if another mechanism is contributing to the retention. To investigate further, a mixture of 3-hydroxybenzoic acid, 3,5 dihydroxybenzoic acid, and 3,4,5 trihydroxybenzoic acid was injected. Surprisingly, the least polar analyte; 3-hydroxybenzoic acid was the first to elute followed by 3,5 dihydroxybenzoic acid, followed by 3,4,5 trihydroxybenzoic acid which is the most polar. This order clarifies that HILIC is also involved in the separation process.

It is also worth mentioning that uracil, which normally elutes unretained in the reversed-phase mode was retained to a greater extent than 3-hydroxybenzoic acid, further demonstrating the presence of hydrophilic interactions between polar analytes and the PEO patches on the stationary phase. To determine the predominant mode, the effect of mass fraction of acetonitrile (%ACN) in the mobile phase on the retention time was studied using toluene and 3-hydroxybenzoic acid; an inconsistent change in the retention time was obtained when %ACN was increased which again indicates the existence of RP/HILIC mixed mode. It is important to mention here that the elution order of the two analytes was reversed at high %ACN. That means reversed-phase was dominant at low %ACN while HILIC was predominant at high %ACN. The mechanical stability and efficiency of the column were also studied. The column was stable to the increasing flow rates up to 3.0 μL min⁻¹ using aqueous and organic mobile phases. This high permeability enables for increasing the column length and allows for further modification of the column.

As an example of applying this material to the separation of small molecules, a mixture of three analytes (3-hydroxybenzoic acid, 2,6-dichlorophenol and pentachlorophenol) was injected using 20%ACN. As shown in Fig. 11-B, a reasonable separation was obtained which proves that this type of stationary phases could be promising for various applications of chromatographic retention, especially under mixed mode.

Conclusion
PEO-based, brush-like amphiphilic macro-RAFT agent with a specific HLB value act in the same fashion as common surfactants for the stabilization of oil in water emulsions. As a result, these polymeric stabilizers can be used as a sole stabilizer of an inverse HIPE system to directly prepare hydrophilic polyHIPEs, consisting of cross-linked acrylamide in the continuous phase. The innovative nature of this approach is further illustrated by the high degree of spatial control for placement of functionalities within the monolithic structure. We identified important parameters to take into consideration for in situ polymerization of polyHIPE within a capillary column. Furthermore, these columns were investigated as stationary phase for high-performance liquid chromatography. Using a nano-liquid chromatography, it has been shown that the polyHIPE are decorated with different microenvironments amongst the voids or domains of the monolithic structure and the result suggests the existence of RP/HILIC mixed mode with promising performance for separation of small molecules. In addition to the applied context of these materials, this work also serves as the first demonstration of the role of the RAFT group of the emulsifier in the attachment of the obtained polyHIPE to the column surface.

Experimental Section
Materials. Poly (ethylene glycol) methyl ether acrylate (PEO MA, average Mn ≈ 480) was purchased from Sigma-Aldrich and used as received. Styrene (Sty, Aldrich, 99%) was passed through a column of Al₂O₃ to remove the inhibitor. The RAFT agent, 2-[((butylsulfanyl)-carbonothioyl)sulfanyl]propanoic acid (PABTC), was
Synthesis of PEO-based amphiphilic surfactant by RAFT polymerization. A series of amphiphilic quasi-block macro-RAFT agents (Qb) consisting of PEO MA and Sty were synthesized as reported in the literature. The PEO-based (PEO MA, average Mₐ ≈ 480) was selected as it provides a hydrophilic group to assist the solubility of the macro-RAFT agent in the aqueous continuous phase. A typical polymerization protocol that was adopted is summarized: In first step, 1 g (4.20 × 10⁻² mol) of PABTC and 0.12 g (4.20 × 10⁻⁴ mol) of V501 were introduced to a round-bottom flask and which was then sealed with a rubber septum, and solids were purged with pure argon for 10 min. In a second step, 10.08 g (2.10 × 10⁻² mol) of PEO MA was then dissolved in 100 mL of dioxane before addition to the round-bottom flask to obtain a solution. This was purged with ultra pure argon for 10 min. The reaction was allowed to proceed at 70 °C for 6 h under constant stirring. After quenching the reaction in an ice bath, a small aliquot of the solution was removed for ¹H NMR analysis to determine the conversion of the PEO MA single block. Styrene and V501 were then added to the round bottom flask at a molar ratio (relative to the initial chain transfer agent concentration) equal to the desired number of monomer repeat units per macro-RAFT agent. The mixture was purged with ultra pure argon for 10 min and further polymerization for 12 h at 70 °C was performed. After which a small aliquot of the solution was removed for SEC and ¹H NMR analysis. The degree of polymerization of the macro-RAFT agent was determined by ¹H NMR spectroscopy. Dioxane was then removed through rotary evaporation under reduced pressure and all polymers were purified by dialysis. Dialysis involved placing the polymer into dialysis tubing (MWCO 2000) and then submerging the polymer and tubing in deionised water (DI) with agitation (see Supporting Information Figure S10). Water was then removed via freeze-drying of the macro-RAFT agents at −30 °C under reduced pressure for at least 100 hours. The polymer was then stored at 4 °C until use. Figure S11 shows the NMR spectra of RAFT-PEO MAₙ-b-Styₘ (Table 1).

Synthesis of hydrophilic ‘inverse’ polyHIPEs. The macro-RAFT agent was dissolved in 4 mL of water without any adjustment of pH. Toluene (16 mL) was added drop-wise to an aqueous solution of macro-RAFT agent with a desired concentration; at a rate of 0.8 mL min⁻¹ with constant stirring at 1000 rpm. The emulsion was stirred for an additional 20 min after complete addition of the internal toluene phase. The drop test method was used to determine the type emulsion prepared and optical microscopy was used to examine emulsion stability. Prior to the polymerization, fused silica capillaries with different internal diameters were modified with 3-(trimethoxysilyl)propyl methacrylate (γ-MAPS) using a procedure previously described (see Supporting Information Figure S14). Using an ice bath to retard the polymerization reaction, an inverse HIPE was introduced to the capillary column using pressure of nitrogen (see Supporting Information Figure S15). In situ polymerization of an inverse HIPE in a capillary was conducted using a KPS/TEMED redox couple as initiator. In situ preparation of hydrophilic polyHIPE columns. A capillary format was chosen as a ‘column housing’ for poly(AAM-MBAM) based hydrophilic polyHIPE to be evaluated as stationary phases for nano-LC. Prior to the polymerization, fused silica capillaries with different internal diameters were modified with 3-(trimethoxysilyl)propyl methacrylate using a procedure previously described (see Supporting Information Figure S14). Using an ice bath to retard the polymerization reaction, an inverse HIPE was introduced to the capillary column using pressure of nitrogen (see Supporting Information Figure S15). In situ polymerization of an inverse HIPE in a capillary was conducted using a KPS/TEMED redox couple as initiator. The chemical stability and swelling behavior of monolithic columns. The chemical stability of polyHIPEs in capillary formats was described by pressure drop of monolithic columns at different flow rates using pure water and acetonitrile as mobile phase. For each flow rate, the pressure values of the HPLC system were measured without and with the column, and the pressure drop across the monolith was calculated as the difference between these two values. Characterization. NMR analyses was performed on a Bruker Ultra Shield Avance Spectrometer (600 MHz). For all NMR analyses deuterated solvents were used as stated. Size exclusion chromatography (SEC) was performed with a Viscotek instrument using refractive index detector (RID) and two chromatography columns (two PSS S linear 3 μm, Polymer Standard Services GmbH, PSS), THF (HPLC grade) was used as an eluent at a flow rate of 0.5 mL/min. The column oven was kept at 40 °C. The calculated molecular weights were based on a calibration curve for polystyrene (PSty) standards of narrow polydispersity with a molecular weight range of 160–154000 g/mol (PSS-Polymer Laboratories). The standards were prepared and injected, the column injection volume was 0.1 mL. Emulsion droplets were observed by optical microscopy (Nikon, model Eclipse E200), equipped with a camera (Tucson, model IS500). Images of the emulsions were analyzed by ImageJ (NIH image). From these, the...
mean droplet size and size distribution were evaluated. Three samples were analyzed for each experiment and the reported results are the average of these. More than 100 droplets were measured. PolyHIPEs were characterized by field emission gun scanning electron microscopy (FE-SEM) studies using a Hitachi SU-70 FESEM in the Central Science Laboratory, University of Tasmania. All samples were plated on a silicon wafer and sputter coated with gold (Emitech 550, Emitech Ltd., UK). The composition of the material was examined by EDX experiments where the materials were sputter-coated with carbon (Ladd 40000 carbon evaporator) before analysis. The calculation of the average pore and windows diameter was performed on sets of at least 100 pores and 100 windows, respectively, using the image analysis software ImageJ (NIH image). A statistical correction was employed to obtain more accurate value, as each value was multiplied by 2/(3−1)2 as described by Carnachan et al.8,9.

The sulfur content of the polyHIPEs was determined with a Thermo Finnigan EA 1112 Series Flash Elemental Analyser. Thermogravimetric analyses were carried out using Setaram LABSYS Evo TG-DSC Thermogravimeter in the temperature range from 30 to 600 °C at the heating rate of 5 °C min−1 under nitrogen atmosphere. The sample mass was about 15 mg. FTIR spectra were recorded by a Bruker Vertex 70 infrared spectrometer equipped with an ATR probe. TheBrunauer–Emmett–Teller (BET) surface area and microporosity were assessed using a Tristar II analyzer for the nitrogen adsorption/desorption at 77 K (Particle and Surface Science, Gosford, AUS). The nano-liquid chromatography studies were performed using an Ultimate 3000 RSLCnano system (Dionex, Sunnyvale, CA). A 1μL sample loop was used and the system was operated with Chromeleon software. UV absorbance was monitored at 214 nm.

Data availability statement. The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References
1. Wu, D. et al. Design and preparation of porous polymers. Chem. Rev. 112, 3959–4015, doi:10.1021/cr200440v (2012).
2. Bartle, H. & v. Bonin, W. Über die Polymisation in umgekehrter Emulsion [On the polymerisation in reversed emulsions]. Makromol. Chem. 57, 74–95, doi:10.1002/macp.1962.020570105 (1962).
3. Bartle, H. & v. Bonin, W. Über die Polymisation in umgekehrter emulsion: II [On the polymerisation in reversed emulsions]. Makromol. Chem. 66, 151–156, doi:10.1002/macp.1963.020660115 (1963).
4. Lissant, K. J. & Mayhan, K. G. A study of medium and high internal phase ratio water/polymer emulsions. J. Colloid Interface Sci. 42, 201–208, doi:10.1016/9797(73)90025-8 (1973).
5. Barby, D. & Haq, Z. Low density porous cross-linked polymeric materials and their preparation. Macromolecules 14, 1370–1377, doi:10.1021/ma035944y (2004).
6. Williams, J. M. High internal phase water-in-oil emulsions: influence of surfactants and cosurfactants on emulsion stability and foam quality. Langmuir 7, 1370–1377, doi:10.1021/la00055a014 (1991).
7. Barbetta, A. & Cameron, N. R. Morphology and Surface Area of Emulsion-Derived (PolyHIPE) Solid Foams Prepared with Oil-Phase Soluble Porogenic Solvents: Three-Component Surfactant System. Macromolecules 37, 3302–3313, doi:10.1021/ma050944y (2004).
8. Kircher, L., Theato, P. & Cameron, N. R. In Functional Polymers by Post-Polymerization Modification 333-332 (Wiley-VCH Verlag GmbH & Co. KGaA, 2012).
9. Kovacic, S., Kren, H., Krajnc, P., Koller, S. & Slušovec, C. The use of an emulsion templated microcellular poly(dicyclopentadiene-co-norbornene) membrane as a separator in lithium-ion batteries. Macromol. Rapid Commun. 34, 581–587, doi:10.1002/marc.201200754 (2013).
10. Shirshova, N. et al. Polymeric high internal phase liquid-in-oil emulsions as potential separators for lithium ion batteries. J. Mater. Chem. A 1, 9611–9619, doi:10.1039/c3ta10856b (2013).
11. Asfaw, H. D. et al. Nanosized LiFePO4-decorated emulsion-templated carbon foam for 3D micro batteries: a study of structure and electrochemical performance. Nanoscale 6, 8804–8813, doi:10.1039/c4nr01682c (2014).
12. Zhao, C., Danish, E., Cameron, N. R. & Kataky, R. Emulsion-templated porous materials (PolyHIPEs) for selective ion and molecular recognition and transport: applications in electrochemical sensing. J. Mater. Chem. 27, 2446–2453, doi:10.1039/b700929a (2017).
13. Nalawade, A. C. et al. Inverse high internal phase emulsion polymerization (i-HIPE) of GMMA, HEMA and GDMA for the preparation of superporous hydrogels as a tissue engineering scaffold. J. Mater. Chem. B 4, 450–460, doi:10.1039/c5tb01873k (2016).
14. Hayward, A. S., Sano, N., Przyboroski, S. A. & Cameron, N. R. Acrylic-Acid-Functionalized PolyHIPE Scaffolds for Use in 3D Cell Culture. Macromol. Rapid Commun. 34, 1844–1849, doi:10.1002/marc.201300709 (2013).
15. Zhou, S., Bismarck, A. & Steinke, J. H. G. Ion-responsive alginate based macroporous injectable hydrogel scaffolds prepared by emulsion templating. J. Mater. Chem. B 1, 4736–4745, doi:10.1039/c3tb02888e (2013).
16. Oh, B. H., Bismarck, A. & Chan-Park, M. B. High internal phase emulsion templating with self-emulsifying and thermoresponsive chitosan-graft-PNIPAM-graft-oligophosphate. Biomacromolecules 15, 1777–1787, doi:10.1021/bm500172a (2014).
17. Liu, H., Wan, D., Du, J. & Jin, M. Dendritic Amphiphile Mediated One-Pot Preparation of 3D Pt Nanoparticles–Decorated PolyHIPE as a Durable and Well-Recyclable Catalyst. ACS Appl. Mater. Interfaces 7, 20885–20892, doi:10.1021/acsami.5b06283 (2015).
18. Pulko, I., Kolar, M. & Krajnc, P. Atrazine removal by covalent binding to piperoxane functionalized PolyHIPEs. Sci. Total Environ. 386, 114–123, doi:10.1016/j.scitotenv.2007.06.032 (2007).
19. Hus, S., Kolar, M. & Krajnc, F. Separation of heavy metals from water by functionalized glycylid methacrylate poly (high internal phase emulsions). J Chromatogr A. 1437, 168–175, doi:10.1016/j.chroma.2016.02.012 (2016).
20. Du, F. et al. High-internal-phase-emulsion polymeric monolith coupled with liquid chromatography-electrospray tandem mass spectrometry for enrichment and sensitive detection of trace cytokinins in plant samples. Anal. Bioanal. Chem. 407, 6071–6079, doi:10.1007/s00216-015-7872-3 (2015).
21. Tebboth, M., Kogelbauer, A. & Bismarck, A. Effectiveness of Emulsion-Templated Macroporous Polymer Micromixers Characterized by the Bousse Reaction. Ind. Eng. Chem. Res. 54, 5974–5981, doi:10.1021/acs.iecr.5b03493 (2015).
22. Tebboth, M., Kogelbauer, A. & Bismarck, A. Liquid–Liquid Extraction within Emulsion Templated Macroporous Polymers. Ind. Eng. Chem. Res. 54, 7284–7291, doi:10.1021/acs.iecr.5b01346 (2016).
23. Barlik, N., Keskınlı, B., Kocakerim, M. M. & Akay, G. Surface modification of monolithic PolyHIPE Polymers for anionic functionality and their ion exchange behavior. J. Appl. Polym. Sci. 132, doi:10.1002/app.42286 (2015).
24. Arrua, R. D., Causon, T. J. & Hilder, E. F. Recent developments and future possibilities for polymer monoliths in separation science. Analyst. 137, 5179–5189, doi:10.1039/c2an203804b (2012).
25. Cameron, N. R. High internal phase emulsion templating as a route to well-defined porous polymers. Polymer 46, 1439–1449, doi:10.1016/j.polymer.2004.11.097 (2005).
Acknowledgements
This work was supported by the Australian Research Council’s Discovery funding scheme (DP130101471). The
acknowledge the Australian Commonwealth Government for an International Postgraduate Research Scholarship
(IPRS) awarded to A.KH. We gratefully acknowledge Dr. James Horne for assistance with NMR, Dr. Karsten
Gömann and Dr. Sandrin Feig for assistance with scanning electron microscopy (Central Science Laboratory,
University of Tasmania). We also gratefully acknowledge Dr. Florencia Torres and Mr. Chris Desire (University of
Tasmania) for assistance with preparation of polyHIPEs within capillary columns.

Author Contributions
The study was conceived and designed by A.K. and E.F.H. Experiments were conducted by A.K. and F.R.M. Data
was analyzed by A.K. and F.R.M with the support of S.C.T. and R.D.A. The first draft of manuscript was written
by A.K. and all the authors reviewed and revised the manuscript to its final form. The entire study was carried out
under the supervision of R.D.A., S.C.T. and E.F.H.

Additional Information
Supplementary information accompanies this paper at doi:10.1038/s41598-017-08423-x

Competing Interests: The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and
institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International
License, which permits use, sharing, adaptation, distribution and reproduction in any medium or
format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Cre-
ative Commons license, and indicate if changes were made. The images or other third party material in this
article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the
material. If material is not included in the article’s Creative Commons license and your intended use is not per-
mitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the
copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2017