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# Polymerized High Internal Phase Emulsion Monoliths for the Chromatographic Separation of Engineered Nanoparticles

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**ABSTRACT:** PolyHIPEs of ethylene glycol dimethacrylate (EGDMA) and styrene/divinylbenzene were prepared by polymerization of water-in-oil high internal phase emulsions (HIPEs) within high pressure liquid chromatography (HPLC) columns. The columns were incorporated into a HPLC system affixed to an inductively-coupled plasma mass spectrometer, and their potential for the separation of engineered nanoparticles was investigated. Triplicate injections of 5 and 10 nm gold particles injected onto a poly(styrene-*co*-divinylbenzene) polyHIPE column produced an average difference in retention time of 135 s. On a poly(EGDMA) column, triplicate injections of dysprosium containing polystyrene particles of 52 and 155 nm produced a difference in retention time of 8 s. In both cases the smaller particles eluted from the column first. Comparison, using scanning electron microscopy, of the polyHIPE columns after the separations, against freestanding monoliths produced from the same HIPEs, revealed no apparent change in the internal porous structure of the polyHIPEs. © 2015 The Authors Journal of Applied Polymer Science Published by Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *132*, 41229.

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## INTRODUCTION

The growth in the use of engineered nanoparticles (ENPs) in consumer products has resulted in an increasing potential for their release into the environment, leading in turn to legislation[1] pertaining to nanomaterials. Agencies, such as the Food and Environment Research Agency (Fera) in the UK, have thus become increasingly concerned with the end-of-life fate of ENPs. Such interest has increased the need for analytical tools capable of separating and identifying nanomaterials, with many approaches being investigated by different researchers.[2] Fera has a particular interest in hyphenated techniques, which combine chromatographic size-based separation with a spectroscopic method of identification. The present work, which was motivated by the need for improved chromatographic separations of nanoparticles, explores the possibility of using monolithic columns based on polymerized high internal phase emulsions (polyHIPEs) as separation media for ENPs.

The first reported use of monoliths as chromatographic separation media can be attributed to Mould and Synge[3] some 60 years ago. Since then, monolithic materials have become commonplace as separation media, frequently used in various forms of high pressure liquid chromatography (HPLC) and in capillary electrochromatography (CEC). Monolithic stationary phases offer good alternatives to packed columns for several reasons: they are relatively easy to prepare,[4] their mechanical strength allows their use in columns without the need for retaining frits (thereby reducing potential analyte/frit interactions),[5] they can have greater relative efficiency than columns packed with beads, because they are not prone to channeling of the analyte solution (the macroporous nature of a monolith forces the analyte solution to pass through its entire structure, resulting in convective mass transfer, which is more rapid than the diffusive mass transfer occurring in packed column systems)[6] and they also tend to exhibit a lower flow resistance, resulting in higher permeability and therefore a faster separation.[4]

For use as a chromatographic support, a polymer monolith should be highly porous, with interconnected pores to permit flow.[5] Monoliths prepared by polymerization of monomer in the presence of a porogenic solvent[6] can (for methacrylates) produce materials with porosities of up to 65%, above which the mechanical stability becomes compromised.[7] Another method that can be employed for the production of porous monoliths is emulsion templating. If a monomer is polymerized in the external phase of an emulsion, the droplets of the

emulsion act as templates for the pores. By using a high internal phase emulsion (HIPE), which is an emulsion with an internal phase volume fraction >74.05%,[8] a porous polymer monolith, termed a polyHIPE, with a network of large pores (referred to in this work as cages) and interconnected by smaller pores (referred to in this work as windows) can be produced.

Initially prepared from styrene and divinylbenzene (DVB), polyHIPEs have subsequently been prepared from a wide variety of monomers.[9] As well as being used in a wide range of applications such as tissue engineering,[10] reaction and catalyst supports[11,12] controlled release matrices[13] and filtration,[14] polyHIPEs have also attracted attention as potential chromatographic stationary phases due to their highly open structures, and nominal pore volumes up to, and sometimes exceeding, 90%.[15] There are patents[16,17] claiming the use of polyHIPEs for a variety of separations. Tunç and co-workers have used both poly(isodecylacrylate-*co*-DVB)[18] and poly(styrene-*co*-DVB)[19] polyHIPE columns for the separation of alkylbenzenes by CEC, reporting good resolution and strong electroosmotic flow, thereby negating the need for an electroosmotic flow-generating monomer. Kovačič and Krajnc[20] polymerized HIPEs containing 4-vinylbenzene chloride inside columns of polyetheretherketone; the resulting polyHIPEs were then functionalized by a flow-through method and the functionalized surfaces of the columns were used for the removal of acid chlorides of a solution pumped through the columns.

In terms of biological separations, polyHIPEs have been investigated for the separation of proteins. Yang and co-workers[21] prepared polyHIPEs from a vinylester resin cross-linked with ethylene glycol dimethacrylate (EGDMA), with which they separated immunoglobin from an aqueous solution of human plasma and chicken egg yolk, as well as separating interleukin-18 and lysine. Yao and co-workers[22] prepared poly(glycidyl methacrylate-*co*-EDGMA) monolithic polyHIPE columns and used ring-opening of the epoxy groups present on the glycidyl methacrylate (GMA) to give the columns weak anion exchange properties. These columns were then used in the separation of a protein mixture of lysozyme, bovine serum albumin (BSA), ovalbumin and pepsin A, with an almost complete separation being achieved at a flow rate of 6 mL $min^{-1}$. Krajnc and co-workers[7] functionalized the epoxy groups of poly(glycidyl methacrylate-*co*-EDGMA) monolithic polyHIPE columns, and then used them to separate myoglobin, conalbumin and trypsin inhibitor, with a good separation being achieved over a short time scale. Pulko and co-workers[23] cast polyHIPE membranes of poly(GMA-*co*-EDGMA-*co*-ethylhexyl methacrylate) which they then modified to form ion-exchange membranes for use in the purification of BSA.

Despite the reported uses of polyHIPEs as chromatographic stationary phases, there are, as far as the authors are aware, no cases of polyHIPEs being used for the separation of ENPs. With the recent interest in the development and use of engineered nanomaterials, there is an urgent need for novel size-based separation and detection methodologies.[24] The two most widely used separation techniques are based on hydrodynamic chromatography (HDC)[25–28] and field-flow fractionation (FFF)[28–30]as they can provide particle sizing data and, more importantly, can be easily interfaced to element-specific detection techniques such as inductively-coupled plasma mass spectrometry (ICP-MS). Fera has, historically, focussed its efforts on HDC because of its ability to deal with a wide range of sample matrices, its low column/analyte interactions (much better on-column recoveries than FFF) and its ability to analyse certain ionic forms of speciated elements. However, as highlighted by Gray et al.,[28] it is restricted by its limited resolution when operating in the low size range, that is, <65 nm. Therefore, in an attempt to address this fundamental shortfall of HDC, the main aim of the study presented here was to develop stationary phases from polyHIPEs capable of the size-based separation of ENPs in the <100 nm range, as part of the progression of previously-reported work utilizing HDC.[25,27] We present data from the development and initial evaluation of two polyHIPE monolithic columns, based on poly(styrene-*co*-DVB) and poly(EGDMA), for the separation of two ENP systems (dysprosium-doped polystyrene particles and citrate-stabilized gold). The two systems reported in this work, poly(styrene-*co*-DVB) and poly(EGDMA), were chosen as the most promising formats for investigation after the following polyHIPE systems had been assessed in previous work:[31] poly(benzyl methacrylate-*co*-EGDMA), poly(butyl acrylate-*co*-EGDMA), poly(butyl methacrylate-*co*-EGDMA), poly(glycidyl methacrylate-*co*-EGDMA), poly(2-hydroxypropyl methacrylate-*co*-EGDMA), poly(methyl methacrylate-*co*-EGDMA), polyEGDMA, poly(styrene-*co*-DVB), and poly(2-hydroxyethyl methacrylate-*co*-N, N′-methylenebisacrylamide).

## EXPERIMENTAL

### Preparation of the PolyHIPEs

**Chemicals.** Styrene (≥ 99%, containing *p-tert*-butylcatechol as an inhibitor; Sigma-Aldrich) and DVB (technical grade, 80%, 1000 ppm *p-tert*-butylcatechol as inhibitor, Aldrich) were distilled, under reduced pressure, to remove inhibitors, and then stored at −20°C until use. EGDMA (98%, stabilized with 100 ppm 4-methoxyphenol, Alfa Aesar) was passed through an alumina column (activated, neutral, Brockmann activity I; Fluka), to remove inhibitors, and then through a 0.45 μm HDPE filter (Millipore) and used immediately. Calcium chloride (anhydrous; Laboratory Reagent, Fisher), Pluronic® L-121 [Poly(ethylene glycol)-*block*-poly(propylene glycol)-*block*-poly(ethylene glycol), average $M_n$ ~4400; Aldrich], potassium persulphate (KPS; 98+ %, Fisher), 2-propanol (laboratory reagent, ≥ 99.5%, Aldrich), SPAN™ 80 (sorbitane monooleate; Fluka), and N,N,N′,N′-tetramethylethylenediamine (TMEDA; 99%, Sigma-Aldrich) were all used as received. Deionized water was used in all polymerizations.

**Polymerization.** The amounts of monomer(s) and internal phase are listed in Table I. The general procedure for the synthesis of the polyHIPEs was as follows: firstly, a stock solution of internal phase was prepared by dissolving calcium chloride (10 g $L^{-1}$) and potassium persulfate (2.5 g $L^{-1}$) in deionized water. The monomer(s) and surfactant were then placed in a three-neck round-bottom flask and mixed using an overhead paddle stirrer (IKA RW20.n) through the central neck. Sufficient aqueous phase to make a 90%, by volume, internal phase (relative to the amount of monomer) HIPE was then placed in an addition funnel in one of the flask side necks, for example, for 3 mL of monomer 27 mL of aqueous phase was added. The addition funnel and the remaining side neck of the round-bottom flask were sealed, by use of a Suba seal, and both phases

**Table I.** Composition and Polymerization Conditions of the HIPEs Used for the Synthesis of the PolyHIPEs

| PolyHIPE | Monomers | Surfactant | Initiator system | Internal phase volume | Polymerization conditions |
|---|---|---|---|---|---|
| PS-C [a] & PS-M [b] | Styrene (1.4 mL) DVB (0.6 mL) | Span 80 (0.6 g) | KPS | 18 mL | 48 hrs @ 65 ° C |
| EGDMA-C [a] & EGDMA-M [b] | EGDMA (4 mL) | Pluronic L121 (1.2 mL) | KPS/TMEDA | 36 mL | 72 hrs @ room temp. |

[a] C: Polymerized in a stainless steel HPLC column.
[b] M: Polymerized in a glass mould.

were purged with nitrogen (30 min). Once purging was complete, the nitrogen flow was removed from within the two phases; but was left on so as to maintain a nitrogen-rich atmosphere. The internal phase was then added drop-wise to the monomer phase under constant stirring (1200 rpm). Once addition was complete, stirring was continued (30 min) to ensure complete mixing of the HIPE. At this stage, if the HIPE was prepared from EGDMA, then TMEDA (0.08 mL) was added and the HIPE mixed for an additional 2 min. The HIPEs were then removed from the round-bottom flask and injected into an empty stainless steel HPLC column (column length 150 mm, i.d 4.6 mm; Supelco, Germany) with the excess HIPE placed into a cylindrical glass sample vial (23 mm × 60 mm) as a mould for the 'free-standing' monolith. Both were then sealed, the column with the supplied end fittings and the glass vial with the supplied plastic caps. The columns containing HIPEs of styrene/DVB were placed in an oven at 65°C for 48 h; at this temperature a KPS molecule will decompose into two sulfate radical anions. Small amounts of KPS which had diffused into the external phase from the internal phase decompose, initiating polymerization. The columns containing HIPEs of EGDMA were polymerized at room temperature for 72 h; the added TMEDA reacts with KPS forming a redox initiator system, generating radicals at room temperature.

Once polymerization was complete, the freestanding monoliths were removed from the glass vials, washed by Soxhlet extraction with 2-propanol (24 h) and then dried under vacuum at 50°C. After washing, the freestanding monoliths (EGDMA-M and PS-M) appeared as white solids with a slightly chalky texture. The monolithic columns (EGDMA-C and PS-C) were opened and a thin slice removed from each end with a razor blade to expose the internal pore structure, to allow greater access for the mobile phase. Columns were then re-sealed and connected to a pump (Model 305; Gilson UK) and manometric module (Model 805; Gilson UK). The back pressure limit of the pump was set at 8 MPa and a 50 : 50 mixture of ethanol and water pumped through the column at a flow rate of 0.10 mL $min^{-1}$. After 4 h, the eluent was changed to deionized water (with 0.01% w/v sodium azide as a biocide), which was pumped at 0.10 mL $min^{-1}$ for a further 2 h. Initially, the ethanol/water mixture which was being pumped through the polyHIPEs eluted murky white. Over time, the eluent became clear, which was taken as an indication that the polyHIPE columns had been successfully washed through.

### Structural Characterization of the PolyHIPEs

Samples were sputter-coated for two minutes with gold/palladium prior to visualization using a Phillips XL30 FEG scanning electron microscope (SEM). Average cage and window diameters were calculated by manual measurement of a large number of entities ($\geq$ 100 measurements). Once the average diameter of the cages had been determined, a statistical correction was applied by multiplying the average value measured by SEM by $2/(3^{1/2})$. This was done because simple mean diameters calculated from SEM will tend to underestimate the average diameter, as measurements can be made at any random distance from the cage centre. A more comprehensive explanation, including the derivation of the correction, is given in the reference.[32]

The pore size distributions were also calculated manually from the SEM images. While mercury intrusion porosimetry is commonly used to obtain pore size distributions for polyHIPEs, it only gives values for the windows, whereas SEM allows the determination of size distributions of both the cages and the windows. It has previously been shown by Wang et al.[33] that there is a good agreement between the window size distributions measured by SEM and mercury intrusion porosimetry.

### Chromatography

**Equipment.** Chromatography experiments were performed on a HPLC system comprising a HPLC pump (model 307 Gilson; UK), a manometric module (Model 805; Gilson UK), and a manual injector valve (model 9125 Rheodyne) with a 20 $\mu$L polyether ether ketone (PEEK) sample loop. Detection was by means of an ICP-MS (7500CX; Agilent, UK), the instrument operation and acquisition parameters are detailed in Table II. Data were acquired on a PC and processed using ChemStation software (Agilent, UK).

Separations were performed using the polyHIPE columns described above. As an important aspect of this work was to

**Table II.** Instrumental Set Up of the ICP-MS

| Parameter | Setting |
|---|---|
| RF power | 1600 W |
| Nebulizer identity | Agilent Micromist |
| Nebulizer gas flow carrier gas | 1.09 L $min^{-1}$ |
| Make up gas | 0.27 L $min^{-1}$ |
| Helium collision gas | 4.9 mL $min^{-1}$ |
| Sample depth | 8 mm |
| Isotopes monitored | $^{197}$ Au, $^{162}$ Dy, and $^{163}$ Dy |
| Dwell time (s) | 0.1 s |

**Table III.** Size Data of the Particles Injected onto the PolyHIPE Columns (Data from Refs. 31 and 35)

| | Diameter (nm) | | | | | |
|---|---|---|---|---|---|---|
| | DLS [a] | | DCS [b] | | TEM [c] | |
| Particles | $D_z$ [d] | PDI [e] | $D_w$ [f] | $D_w/D_n$ | $D_n$ [g] | RSD [h] |
| 52 nm REEP | 52 | 0.09 | 49 | 1.01 | 33 | 1.9% |
| 155 nm REEP | 155 | 0.02 | 134 | 1.01 | 117 | 7.8% |
| 5 nm Gold Standard | - | - | - | - | 5.6 | < 15% |
| 10 nm Gold Standard | - | - | - | - | 14.6 | < 8% |
| 20 nm Gold Standard | - | - | - | - | 20.7 | < 8% |

[a] Dynamic Light Scattering.
[b] Differential Centrifugal Sedimentation.
[c] Transmission election microscopy.
[d] *z*-Average diameter.
[e] Polydispersity index (defined as PDI = standard deviation $^2$ /$D_z$ $^2$ ).
[f] Weight-average diameter.
[g] Number-average diameter.
[h] Relative standard deviation.

compare the newly generated columns against HDC, a decision was made that the same mobile phase should be used. This mobile phase was a proprietary aqueous eluent concentrate (Agilent, UK) formulated for use with a PL-PSDA type HDC column, which was diluted to the manufacturer's specification. The exact composition of the mobile phase is unknown, the MSDS implied that it contained sodium azide (presumably as a biocide), sodium dodecyl sulfate (an anionic surfactant), and ethoxylated dodecane-1-ol (presumably acting as a non-ionic surfactant).[34] Although the normal approach to developing a chromatographic separation would be to tailor a bespoke mobile phase so as to best fit the analytes, in this study we were seeking to compare the newly produced columns against a known separation procedure, that is, HDC. Hence, by using the HDC eluent, we sought to ensure that variation in elution behavior was due to differences between the column types rather than factors such as ionic strength, pH or surfactant type/concentration.

The relationships between flow-rate, back-pressure, and pump performance were assessed for each column before any injections of analyte were performed. Ideally, the initial flow-rate would be sufficient to allow the pump to function reproducibly, but not too great to cause compaction of the stationary phase. Therefore, a relatively low back-pressure cut-off limit was set at 8 MPa. The results of these initial stages of the assessment process are presented in the relevant sections. Where the optimal "chromatographic" flow-rate was lower than the natural aspiration rate requirement of the ICP-MS nebulizer (0.24 mL min$^{-1}$, Micromist - Agilent, UK), additional flow was provided by means of a peristaltic pump (Miniplus-2; Gilson, UK), and mixed with the column effluent via a low-volume T-piece, located just before the nebulizer.

**Size Calibrants.** The separation efficiency of the columns was assessed using two sets of size calibrants; dysprosium-doped polystyrene latex particles (52 and 155 nm), which had been produced, in-house, as part of an earlier aspect of this current study (termed Rare-Earth Element Particles—REEPs in the resulting work),[31] and gold nanoparticles (5, 10 and 20 nm) commercially available from BBI International.[35] Table III presents the available size and polydispersity data for the two particle types. Particles were diluted to 1.25% v/v in mobile phase before injection.

**Calculations.** Column resolution, $R_s$, was calculated by Eq. (1).

$$R_s = \frac{2(r_{tB} - r_{tA})}{1.7(W_{1/2A} - W_{1/2B})}, \quad (1)$$

where A and B are the two peaks, $r_t$ is retention time in seconds and $W_{1/2}$ is the width at half height of the peak in seconds.

Peak asymmetry factor, $A_s$, was calculated by Eq. (2).

$$A_s = b/a, \quad (2)$$

where $a$ is the width of the front half of the peak at 10% height in seconds and $b$ is the width of the back half of the peak at 10% height in seconds. Values of $A_s > 2.0$ are considered poor, as above this value the resolution becomes compromised, with values of $< 1.5$ considered ideal.[36]

## RESULTS AND DISCUSSION

### Characterization of the PolyHIPEs

Visual inspection of the HIPEs showed them to be stable, at least over the lifetime of the polymerization, with no phase separation observed between formation and completion of the polymerization. The internal pore structure of the polyHIPEs was assessed by SEM (Figures 1 and 2), revealing a typical polyHIPE structure of cages interconnected by windows. This further shows that the HIPEs were stable for the lifetime of the experiment, allowing the HIPE to template the resulting pore structure. As it was not possible to perform SEM on the polyHIPE material packed in the columns prior to use, a set of free-standing monoliths were prepared from the same HIPEs that were used to produce the columns. Small samples of polyHIPE were also removed from the columns after they had been used for chromatography, and characterized by SEM. The SEM

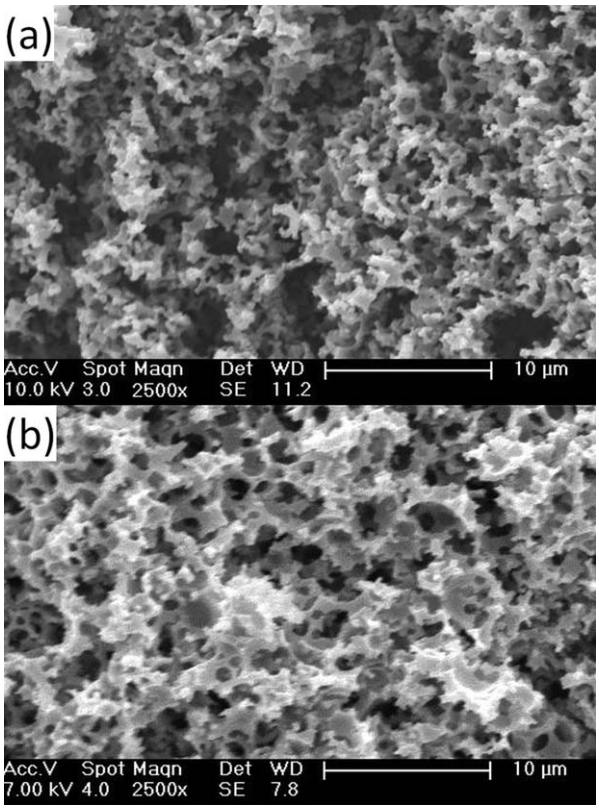


**Figure 1.** SEM images of (a) polyHIPE monolith PS-M, which was not exposed to a chromatographic flow, and (b) polyHIPE monolith PS-C, which had been removed from the chromatographic system after use.

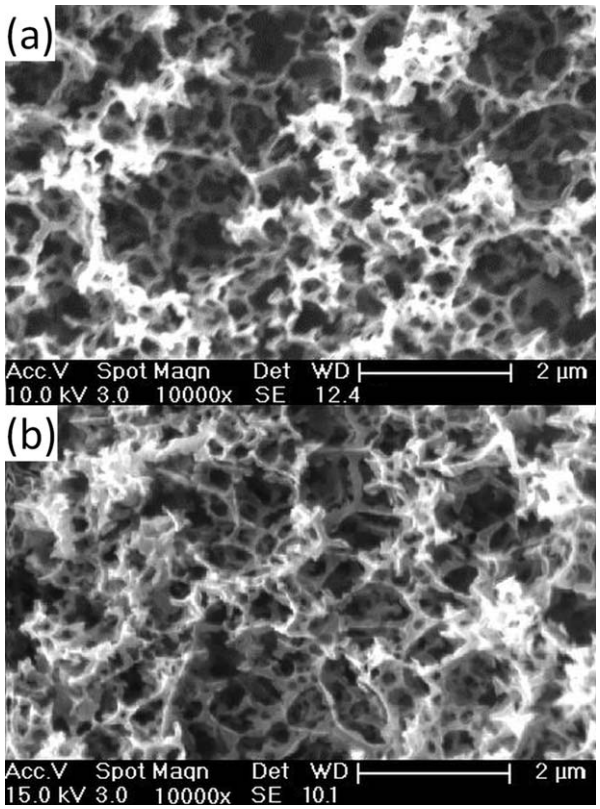


**Figure 2.** SEM images of (a) polyHIPE monolith EGDMA-M, which was not exposed to a chromatographic flow, and (b) polyHIPE monolith EGDMA-C, which had been removed from the chromatographic system after use.

images showed that both the polyHIPEs prepared within the columns and the freestanding polyHIPE monoliths, for both the poly(styrene-*co*-DVB) (Figure 1) and the polyEGDMA (Figure 2) polyHIPEs, were very similar, that is, before and after chromatography. This was further evidenced by the similarity between the average sizes of the cages and windows (Table IV), as well as the corresponding size distributions (Figure 3) of the polyHIPEs. This suggests that, at least in the short term, the polyHIPE structures were sufficiently robust to withstand the pressures associated with the eluent flow, as well as being chemically inert to the mobile phase and the particles separated. While it is possible to quantify the openness of a polyHIPE using the equations proposed by Pulko and Krajnc,[9] for the polyHIPEs described in this work the high levels of openness, in which the windows were not discrete and ran into each other (for both the EGDMA and PS polyHIPEs), prevented reliable application of these calculations.

### Chromatography

**Poly(styrene-co-DVB) PolyHIPE Column (PS-C).** The initial assessment of flow through the poly(styrene-*co*-DVB) column (PS-C) showed that a flow rate 0.5 mL $min^{-1}$ gave a backpressure of $5.4 \pm 0.2$ MPa. PS-C was first investigated for chromatography of REEPs. Unfortunately, after the first injection of the 52 nm REEP, the backpressure increased and exceeded the 8 MPa cut-off limit. The column was reversed, and mobile phase gently pumped through it (0.01 mL $min^{-1}$) overnight. Monitoring $M_z$ 162 ion (associated with dysprosium) showed that the overnight flush had been successful at removing the particles, with the signal returning to the baseline values observed before injection. The mobile phase was then gently pumped in the correct direction again overnight. The backpressure, at 0.5 mL $min^{-1}$ was found to have risen to $7.5 \pm 0.2$ MPa, but was stable. It was clear that even the smallest of the REEPs (52 nm) was too large to transit the column, so the commercially-obtained gold nanoparticles were investigated, as they covered the lower end of the size range (5–20 nm).

Figure 4 presents chromatograms comprised of the mean values obtained from triplicate injections of the 5 and 10 nm gold nanoparticles on column PS-C. The average retention times were $t_r = 353$ s (RSD = 1.5%) for the 5 nm standard and $t_r = 488$ s (RSD = 1.4%) for the 10 nm standard, at the optimal flow rate of 0.3 mL $min^{-1}$, giving a difference in retention time of 135 s. Following on from the injection of 5 and 10 nm gold particles, an injection of 20 nm gold particles resulted in an increased backpressure. The column was again reversed, and the eluent monitored by the ICP-MS. Although only a qualitative observation, the magnitude of the resulting signal (as the retained gold particles were flushed off the column) was much greater than that obtained from the individual injections of the 5 and 10 nm Au-NPs, even though the vial concentration of all three Au-NPs was notionally the same. One explanation is that the column eluent contained not only the 20 nm Au-NPs, but also material retained from the smaller Au-NP systems,

**Table IV.** Mean Cage Diameter, $D_n$, and Mean Window Diameter, $d_n$, of the Poly(styrene-*co*-DVB) and PolyEGDMA Free-Standing (M) and Column-based (C) Monoliths, as Measured by SEM

| | Cage | | Window | |
|---|---|---|---|---|
| PolyHIPE | $D_n$ ($\mu$m) | %RSD | $d_n$ ($\mu$m) | %RSD |
| PS-C | not measurable | - | 0.59 | 37 |
| PS-M | not measurable | - | 0.57 | 42 |
| EGDMA-C | 1.08 | 36 | 0.19 | 57 |
| EGDMA-M | 1.12 | 50 | 0.19 | 50 |

Both formats (M and C) were prepared from the same HIPE, under the same conditions. The SEM was performed on the dismantled monolithic column contents after the chromatography experiments had been performed.

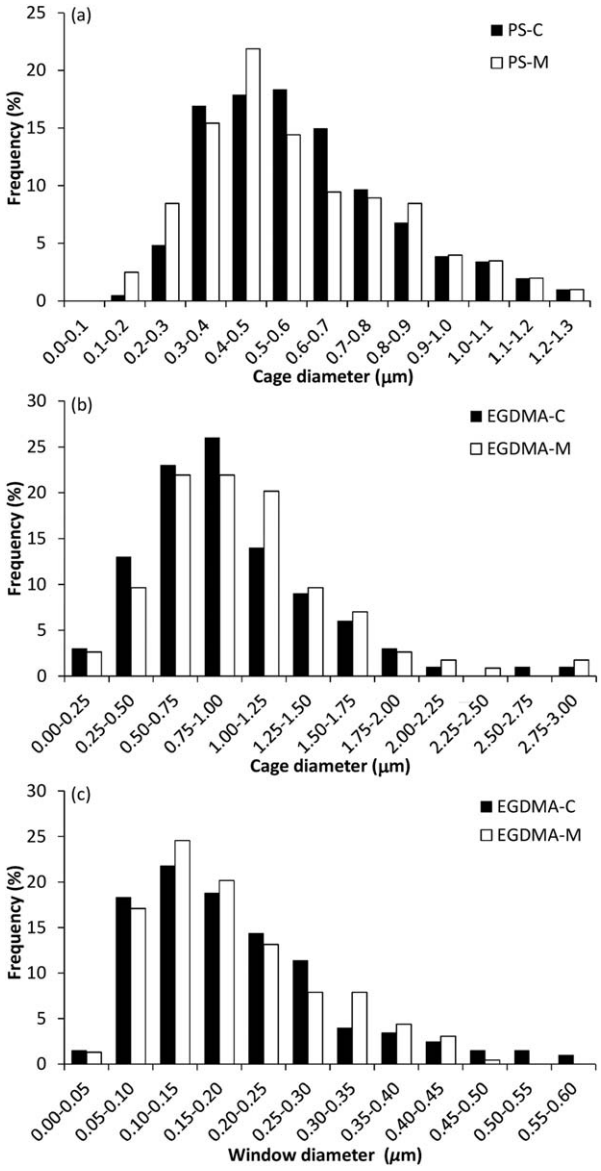


**Figure 3.** Size distributions, determined by SEM, of the cages and windows of polyHIPE monoliths. (a) Distribution of cage diameter of PS-C (after use) and PS-M, (b) distribution of cage diameter of EGDMA-C (after use) and EGDMA-M, and (c) distribution of window diameter of EGDMA-C (after use) and EGDMA-M.

suggesting that, if accurately quantified, on-column losses (even for the smaller particles) would have been significant.

However, encouragingly, there was a significant difference in retention times between the particles injected onto PS-C (135 s). While the peak asymmetry (3.7 for the 5 nm particles and 2.2 for the 10 nm particles) was such that it resulted in less-than-ideal resolution (0.31), the significant difference in retention time might suggest that, either by further development of the column manufacturing process and/or manipulation of the mobile phase, the poly(styrene-*co*-DVB) polyHIPE may have potential as a stationary phase for the separation of ENPs at the lower end of the size range. This is a significant outcome, as one of the commonly quoted limitations of HDC is its inability to resolve peaks below ~65 nm.[26,28]

**Ethylene Glycol Dimethacrylate HIPE Column (EGDMA).** The initial assessment of the flow/backpressure relationship for the column EGDMA-C, at a flow rate of 0.5 mL min$^{-1}$, gave a significantly lower backpressure ($0.8 \pm 0.1$ MPa) than the PS-C column. Figure 5 presents chromatograms comprised of the mean values obtained from triplicate injections of the 52 and 155 nm REEPs on EGDMA-C. The retention times were $r_t = 105$ s (RSD = 2.5%) for the 52 nm particles and $r_t = 113$ s (RSD = 1.4%) for the 155 nm particles, at the optimal flow rate of 0.15 mL min$^{-1}$, giving a difference in retention time of 8 s. From the precision data, it can be seen that, even if only marginal, the separation was reproducible. The optimal flow-rate

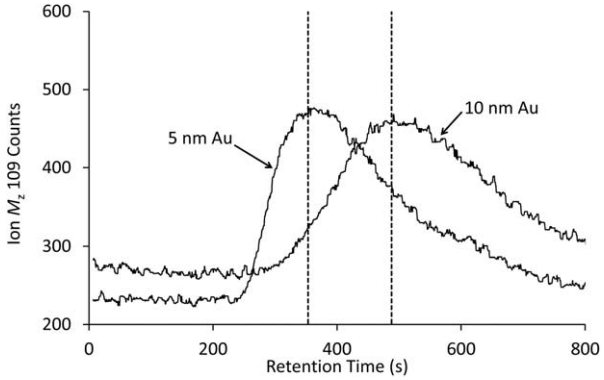


**Figure 4.** Averaged chromatographs of triplicate injections of gold nanoparticles (5 and 10 nm) from the polyHIPE column PS-C. The dashed lines represent the mean retention times of the triplicate injections.

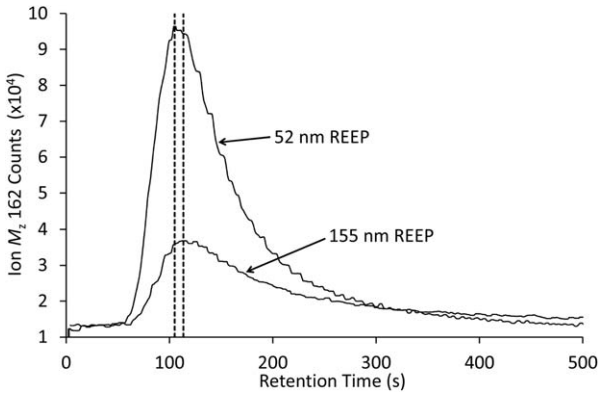


**Figure 5.** Averaged chromatographs of triplicate injections of dysprosium containing REEPs (52 and 155 nm) from the polyHIPE column EGDMA-C. The dashed lines represent the mean retention times of the triplicate injections.

was found to be 0.15 mL $min^{-1}$. However, as this was 0.9 mL $min^{-1}$ below the natural aspiration rate of the ICP-MS nebulizer, there was a significant pulsing of the signal from the ICP-MS. To counteract this, it was necessary to introduce a make-up flow, however this did not completely eliminate the effect, leading to a stepped profile of the peaks (Figure 5).

**Separation Quality Achieved on the PS and EGDMA PolyHIPE Columns.** It was originally envisaged that the mechanism of separation of the polyHIPE columns would be similar to that occurring in HDC or size exclusion chromatography, where the larger particles elute first as a result of their greater excluded volume. However, as the smaller particles eluted first (for both columns) it is clear that mechanisms similar to those in either HDC or SEC were not taking place. It is, at this stage, unclear to what extent, if any, particles interact with the column wall. There could potentially be more than one mechanism in operation and it is therefore uncertain as to whether the separation occurs due to the physical differences in particle size or as a result of chemical interactions between the polyHIPE and the particle surface. Further characterization of the polyHIPE columns to establish in greater detail the physical properties would provide valuable information for developing a viable hypothesis as to the separation mechanism, but this is beyond the scope of the present work.

Both columns produced peaks with less than ideal asymmetry factors, with no values being $< 2.2$, which is indicative of on-column interactions. As the mobile phase was developed for use with HDC, rather than being developed specifically for the individual polyHIPE columns, it is possible that peak asymmetry could be addressed (at least partially) by developing bespoke mobile phases. However, a better understanding of the mechanism by which the separation occurs should enable columns to be developed that minimize the most significant contributions to the observed peak-tailing. In addition to this, it should be remembered that comparable chromatographic "sizing" techniques, such as HDC and SEC, invariably utilize larger columns than used in this work (150 × 4.6 mm). Resolution of the polyHIPE column could, therefore, potentially be improved by scale-up of the column length to that of an HDC column (800 mm). While a theoretical resolution cannot be calculated with any reasonable degree of accuracy, some comment can be made on the potential effects of a column scale-up. For two columns packed with the same stationary phase, the longer one will have a greater number of theoretical plates, and so would be expected to have a better resolution due to the larger number of potential analyte/stationary phase interactions. Also, by increasing the column length, the extra dead volume within the system becomes relatively less significant. However, larger column formats may give rise to greater analyte diffusion, resulting in greater peak broadening, and thereby negating some of the positive aspects.

## CONCLUSIONS

An initial evaluation of the potential of polyHIPE monoliths to act as separation media for nanoparticles has produced some interesting observations. In particular, the peak maxima-to-peak maxima resolution obtained on the poly(styrene-*co*-DVB) polyHIPE column, for 5 and 10 nm gold nanoparticles, is worthy of further investigation, along with further characterization of the polyHIPEs. Separation at this lower end of the nanoparticulate size range is currently one of the biggest challenges faced by researchers in nano-toxicology, given the significance of "size" on the biological and physicochemical properties of these novel products. Hence, a fuller understanding of the mechanisms which brought about the observed separations will be a focus for future work.

Comparison of SEM images of the polyHIPEs after being used for chromatography, against a polyHIPE monolith produced from the same HIPE and polymerized under identical conditions, shows similar micro-scale structures with comparable size distributions for the polyHIPE cages and windows. This indicates that, in the short term at least, the polyHIPE columns are stable and the internal structure is not altered by chromatography.

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