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Effect of ethoxylated sorbitan ester surfactants on the chromatographic efficiency of poly(ethylene glycol)-based monoliths

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Abstract

The effect of adding ethoxylated sorbitan ester surfactants (Tweens®) to poly(ethylene glycol) diacrylate-based monolithic recipes was investigated. Five different Tweens® have been evaluated to investigate the exact role of non-ionic surfactants in poly(ethylene glycol) diacrylate-based monolith preparations. These monoliths were characterized by scanning electron microscopy, infrared spectroscopy, and nitrogen physisorption analysis. Different morphological features, and surface areas were observed when different types of Tween® were included in the recipe; Tween® 20 and 85 showed small globules, while Tween® 40, 60 and 80 exhibited larger globular structures with different sizes and degrees of coalescence. The different Tween®-based monoliths were investigated for the chromatographic

separation of mixtures consisting of hydroxybenzoic acids and alkylbenzenes. These columns were mechanically stable, except for Tween® 80. The highest methylene selectivity and the best overall performance were achieved by Tween® 60. The efficiency was increased by increasing the concentration of the Tween® 60 and the amount of poly(ethylene glycol) diacrylate M_n 700 in the recipes up to 30 wt%, each. Further increases in either Tween® 60 or poly(ethylene glycol) diacrylate M_n 700 led to formation of non-permeable columns. The optimized column was successfully used for separation of mixtures of nonsteroidal anti-inflammatory and sulfa drugs, with a maximum efficiency of 60,000 plates/m.

Keywords: polymer monoliths; porogen; non-ionic surfactants; PEGDA monoliths; pharmaceutical drugs

1. Introduction

Since their introduction in early 1990s, monolithic columns attracted considerable attention from separation experts, due to their high permeability and low resistance to mass transfer [1,2]. According to the chemical nature, monolithic structures can be classified into three main categories: inorganic (mostly silica-based), organic (or polymeric) and hybrid type monoliths [3]. Polymer-based monolithic columns have been widely accepted for various applications in different matrices [4,5]. Compared with inorganic monoliths, the polymeric monolithic columns are preferable in various aspects including the high pH stability, the simple, straightforward procedures used in the preparation and the several variables (type and ratio of monomers, cross-linkers, polymerization conditions, and post-polymerization modification) that can be adjusted to customize the monolith properties.

One of the main challenges in monolith preparation is porogen selection and optimization [5–7]. Changing the porogen type has evident effect on monolith surface area, morphology, pore size distribution, mechanical stability, permeability, and chromatographic efficiency [8,9]. The results of changing porogens are not always predictable, which makes new monolith preparation appear time consuming and difficult. This fact makes porogen selection a tedious step, especially when new monomers, new cross-linkers or new monomer/cross-linker ratios are to be introduced.

A few attempts have been made to try new materials as porogens including gases [10], solids [11] and liquids where the later are the most commonly employed due to ease

of use. Recent reports tried new chemical classes such as ionic liquids [12], polymers [13], and surfactants [14] as liquid porogens. The results of using surfactant porogens were interesting, and were employed in the preparation of both silica- and polymeric-based monoliths [15,16]. The incorporation of polyoxyethylene surfactants in the monolith recipe induced observable changes in morphology, particularly the pore structure [17]. Both homemade [18] and commercially available surfactants [19] were tested as co-porogens. These recipes could overcome the limited solubility problems of monomers [19] and helped to form monoliths with some degree of mesoporosity [16,20]. These monoliths were successfully used for separation of both small and macromolecules in different chromatographic modes including size exclusion [18,20] and reversed phase [16].

In our previous work, we explored the possibility of using a surfactant-containing quaternary mixture as a universal porogen for the preparation of polymer monoliths [21]. The porogenic system consisted of 30% Tween® 40, 20% decane, 15% decanol, and 15% dodecanol and it was successfully applied for preparation of various porous polymer monoliths including poly(hydroxyethyl methacrylate-*co*-ethyleneglycol dimethacrylate), poly(benzyl methacrylate-*co*-EDMA), poly(glycidyl methacrylate-*co*-EDMA), poly(butyl methacrylate-*co*-EDMA), poly(lauryl methacrylate-*co*-PEGDA), poly(bisphenol diacrylate-*co*-PEGDA), poly(N,N-dimethylacrylamide-*co*-PEGDA) and poly(styrene-*co*-divinylbenzene). The maximum chromatographic efficiency was 30,500 plates/m^2 observed for a PEGDA based monolith, using Tween® 40 as a co-porogen.

In this work, we study the effect of the type of sorbitan ester surfactants (Tweens®) on the morphology and chromatographic efficiency of PEGDA monoliths for the separation of small molecules. Five different polysorbitan esters were utilised to investigate the role of non-ionic surfactants in monolith preparation and the effect of chemical structure on the chromatographic performance of PEGDA-based columns. To the best of our knowledge, the use of polysorbate surfactants as co-porogens has not been previously explored. This work aims to improve the chromatographic efficiency of PEGDA monoliths using other polysorbate surfactants.

2. Experimental

2.1 Material

The materials used were of analytical grade, utilized as received except for poly(ethylene glycol) diacrylate (PEGDA M_n 700) and 2,2′-Azobis(2-methylpropionitrile) (AIBN). Decane (≥99%), 1-decanol (~99%), 1-dodecanol (>99%), PEGDA M_n 700, sodium hydroxide (≥98%), sodium sulfate (anhydrous) (≥99.0%), 3-(trimethoxysilyl)propyl methacrylate (≥98%), 4-hydroxybenzoic acid (4-HBA), 2,3-dihydroxybenzoic acid (2,3-DHBA), 2,4-dihydroxybenzoic acid (2,4-DHBA), 3,5-dihydroxybenzoic acid (3,5-DHBA) and the different Tweens® were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetone (>98%), ethanol (>99%) and methanol (≥99.7%) were obtained from Chem-Supply (Gillman, SA, AUS). 2,2′-Azobis(2-methylpropionitrile) (AIBN) was purchased from MP Biomedicals (Santa Ana, CA, USA). Dichloromethane (>99%) was obtained from Unilab (Mandaluyong

City, Philippines). Hydrochloric acid (37%), sodium carbonate (anhydrous) (≥99.9%) benzoic acid (BA), 3-hydroxybenzoic acid (3-HBA), 3,4,5-trihydroxybenzoic acid (3,4,5-THBA) were obtained from Merck (Kilsyth, VIC, Australia). The pharmaceutical drugs were kindly supplied by Pfizer (St. Louis, MO, USA). AIBN was used after being recrystallized from methanol, and kept in the freezer at -4ºC. PEGDA M_n 700 was purified as described in section 2.3 and stored at 5ºC.

2.2 Instrumentation

The chromatographic separations were conducted on an UltiMate™ 3000 RSLC nano system (Dionex, Sunnyvale, CA, USA) that contained an NCS-3500RS capillary LC gradient pump, a membrane degasser unit and an integrated column compartment. The system was also equipped with a VWD-3400RS UV detector, with a 45 nL flow cell and a WPS-3000TPL RS autosampler fitted with a 200 nL sample loop. The system was controlled using Chromeleon® software (Ver. 6.80). The obtained chromatograms were converted to ASCII files and replotted through Origin® 8.5 (Northampton, MA, USA). A Hitachi SU-70 field emission scanning electron microscope (Hitachi, Pleasanton, CA, USA) was operated in high vacuum mode with an acceleration voltage of 1 to 15 kV to obtain the SEM images. Specific surface areas were determined by the Brunauer–Emmet–Teller (BET) method [22] using a Micromeritics Tristar II 2020 automated nitrogen sorption–desorption instrument. Prior to analysis, all samples were dried in a Micromeritics SmartPrep at 70°C for 24 h.

2.3 Purification of PEGDA M_n 700

To remove impurities, and purify PEGDA M_n 700, the liquid polymer was extracted as indicated by Liu et al. [23]. 15 mL of a saturated aqueous solution of sodium carbonate was placed in a 100 mL separating funnel, mixed with 25 mL of PEGDA M_n 700 and vigorously shaken. The funnel was left to allow for phase separation. The lower layer (the carbonate solution) was then discarded and the washing procedures were repeated twice, followed by rinsing with 25 mL of deionized water. Dichloromethane was then added in three aliquots (25 mL each) to extract the PEGDA from the remaining water layer and the lower layer was combined and dried with anhydrous sodium sulfate, filtered through Whatman cellulose-based filter paper (Maidstone, Kent, UK) and the dichloromethane was removed using a rotary evaporator.

2.4 Preparation of monolithic columns

The polyimide-coated 150 μm ID (360 μm od) capillaries (Polymicro Technologies, Phoenix, AZ) were surface modified according to a procedure by Rohr et al. [24]. Briefly, capillaries were flushed with acetone and water, before being activated with 0.2 M sodium hydroxide at 30 $\mu L.h^{-1}$ for 30 min using a syringe pump. The capillary was then rinsed with water, and neutralized with 0.2 M HCl pumped at the same flow rate for 30 min, followed by flushing with water and ethanol at pH 5 (adjusted using acetic acid). The activated surface was then modified by pumping a solution of 20 wt% 3-(trimethoxysilyl)propyl methacrylate in ethanol at pH 5 through the capillary for 1 h at 30 $\mu L.h^{-1}$. The capillary was finally rinsed with acetone and

purged with nitrogen for 2 min before being stored at room temperature for at least 24 h.

The modified fused silica capillaries were then employed as a housing to prepare PEGDA M_n 700 monoliths *in situ* by thermal polymerization. The polymerization mixture consisted of a combination of monomer, porogen, and initiator. The optimized monoliths used in separation were prepared from a solution consisting of 0.3 g PEGDA M_n 700 (30wt%), 0.30 g Tween® 60 (30wt%), 0.16 g decane (16wt%), 0.12 g decanol(12wt%), 0.12 g dodecanol (12wt%) and 0.003 g of AIBN. The mixture was first sonicated in an ultrasonic bath for 10 min, deoxygenated for 5 min using nitrogen, and used to fill the surface modified capillaries. The two ends of the capillaries were sealed and immersed in a water bath at 60°C for 20 h to initialize the polymerization reaction. Any excess polymerization mixture was collected in 4 mL screw-top glass vials, and immersed in the water bath at the same temperature and for the same duration to obtain bulk materials for characterization. After polymerization, bulk monoliths prepared in the vials were cut into smaller pieces and a Soxhlet apparatus filled with methanol was used for 48 h to extract residual porogens. The monoliths were dried at 30°C in a vacuum oven for two days. As for the prepared capillary columns, methanol was used for rinsing at 2.00 $\mu L.min^{-1}$ for 1 h at 40°C using the LC system.

2.5 Capillary liquid chromatography

The LC separations were performed in the reversed phase mode using mobile phases consisting of varying ratios of ACN/water or 0.1% formic acid in ACN

(pH=3.0)/0.1% formic acid in water (pH=3.0) at 2 μL.min^{-1}. The UV detector was operated at 214 nm, and the autosampler was set to deliver 50 nL per injection. The mobile phase was prepared in Milli-Q water (18 MΩ cm, Millipore, USA) and filtered with 0.45 μm cellulose membranes (Grace Davison Discovery Sciences, VIC, Australia) before being sonicated.

2.6 Calculations

Darcy's law [11] was used for permeability (K) calculations as follow:

$$K = \frac{F \times \eta \times L}{\Delta P \times \pi \times r^2}$$

where F is the flow rate (m^3.s^{-1}), η is the mobile phase viscosity (Pa.s), L is the capillary length (m), ΔP is the back pressure (Pa), and r is the inner radius of the capillary (m). The dynamic viscosity of water, used in all the calculations was 0.798×10^{-3} Pa.s at 30°C.

Martin's rule [25,26] was used to calculate the methylene selectivity (α) using the following equation:

$$\log k = n_C \log \alpha + \log \beta$$

Where k is the retention factor of a group of compounds that differ only by one methylene group, β is the calculated retention factor of the parent compound, $n_C = 0$ (in this case, benzene was the parent compound which does not have any alkyl substituents). When Log$k$ was plotted against nc, a straight line was obtained and the slope was used to calculate the methylene selectivity. The number of theoretical plates (N) was also

185 calculated using the following equation:

186 $$N = 16(\frac{t_r}{w_b})^2$$

187 where t_r is the retention time of gallic acid using a mobile phase consisting of
188 ACN/water (20:80, v/v) at 1.0 mL.min^{-1} and w_b is the peak width at 13.4 % of the peak
189 height.

190 **3. Results and discussion**

191 In our previous attempts to introduce a universal porogenic mixture that can be
192 used for different monolith recipes [21], Tween® 40 showed the best performance. In
193 this work, Tween® 40 was replaced with other types of Tweens® of different chain
194 length, degrees of substitution and saturation. **Fig. 1** summarizes the differences
195 between the Tweens® employed in this study. Each Tween® was mixed with PEGDA
196 M_n 700, decane, decanol, dodecanol and AIBN and heated at 60°C for 20 h in a water
197 bath, as summarized in **Table 1**. SEM was used to characterize the formed polymers;
198 as shown in **Fig. 2**, the five different Tweens® gave regular monolith structures with
199 significantly different morphologies. The monoliths prepared with Tween® 20 and 85
200 possessed small globules, while those prepared with Tween® 40, 60 and 80 exhibited
201 larger globular structures with different sizes and degrees of coalescence. On the
202 other hand, a heterogeneous polymer was obtained when no surfactant was added to
203 the polymerization recipe (C0); nonporous chunks were observed in the bulk material
204 except at the surface, where spherical particles were formed (**Fig. S1**). These results

highlight the importance of adding polysorbate surfactants to the porogenic mixture.

To investigate the presence of surfactant after the washing step, FT-IR spectra of different monoliths were recorded. As shown in **Fig. S2**, three characteristic peaks of PEGDA monolith were observed at 1100, 1730, 2875 cm^{-1}. These peaks correspond to stretching vibrations of C−O, C=O and aliphatic C−H bonds, respectively. No significant differences were observable between the FT-IR spectra of C0 and each of the five columns C1-C5. This illustrates that the surfactants were not incorporated in a detectable amount in the formed polymer, which confirms that these ester compounds served only as part of the porogen mixture and were washed out after the polymerization reaction.

The mostly nonporous structure obtained in C0 when no surfactant was added, could be explained if the three solvents used in the porogenic mixture (decane, decanol and dodecanol) were all poor solvents [8]. The spherical particles formed at the surface of C0 may be due to the very small amount of PEGDA M_n 700 that has been dissolved in the mixed poor solvents at the interface. That was confirmed by the small BET surface area of C0 compared with the other columns (**Table 1**). On the other hand, increasing the chain length of the surfactant led to an increase in the BET surface area where laurate-substituted Tween® (Tween® 20) was less than palmitate (Tween® 40), less than stearate (Tween® 60). The presence of unsaturated groups was also accompanied by an increase in the surface area as shown in Tween® 80 and Tween® 85. The suitability of these different columns for chromatographic separation in 150 µm ID

was evaluated. C4 that was prepared using Tween® 80 was mechanically unstable and detached from the functionalized wall as shown in **Fig. S3**. The other columns, C1-C3 and C5 that has been prepared by the other surfactant with unsaturated branches, were mechanically stable. This difference in stability between C4 and C5 can be attributed to the higher degree of substitution of Tween® 85 (Polyoxyethylenesorbitan trioleate) compared to the mono-oleate form; Tween® 80.

3.1 Separation of small molecules

The applicability for small molecule separation was evaluated by investigating the separation of hydroxy benzoic acid derivatives in the reversed phase mode. The observed elution order (**Fig. 3**) was not typical for reversed phase mode, which indicated that some additional interactions were contributing to the retention mechanism. The H-bonding between the hydroxyl groups of the analytes and the polar ethylene glycol chains of PEGDA M_n 700 could explain the unexpected elution behaviour [14]. Increasing the %ACN was associated with a decrease in the retention time of all analytes, which suggests that the separation was performed in the reversed phase mode. As shown in **Fig. S4**, Tween® 20 showed the lowest retention and the worst resolution. 3-HB and 4-HBA were better resolved on C2 and C3, while C5 gave higher resolution for 3,5-DHBA and 3,4,5-THBA. The best overall chromatographic separation was observed with C3. The methylene selectivity was calculated using a mixture of toluene, ethyl-, propyl-, butyl- and pentylbenzene. As shown in **Table 1**, the highest selectivity was achieved by C3 and C5. The columns efficiencies were

measured using gallic acid (*k'*=5.1) and a mobile phase consisting of ACN/water (20:80, v/v). The highest efficiency was achieved with C3. Thus, Tween® 60 was chosen for further optimization.

3.2 Optimization of the surfactant concentration

To additionally optimize the monolith for small molecule separations, different concentrations of the Tween® 60 were employed to prepare column C6-10, as summarized in **Table 2**. As shown in **Fig. 4**, using 10wt% of Tween® 60 (as in C6) led to the formation of monolith with macroglobules that looked similar to the spherical particles formed at the interface of the control monolith (C0). Increasing the amount of Tween® 60 from 10 to 35wt% led to an observable change in the microglobule shapes with a corresponding increase in the surface area (**Table 2**). Upon trying the prepared monoliths for chromatographic separation, C9 that was prepared using 30wt% of the surfactant had the highest efficiency. Using higher concentrations of Tween® 60 was accompanied with higher surface area, but the formed monolith was not practically permeable. Increasing the surfactant concentration did not change the role of Tween® in the monolith recipe; the performed FT-IR for the columns C6-10 were identical coinciding with the control column as expected. **Table 2** summarizes the differences in surface area, efficiency, and permeability between columns (C6-C10).

3.3 Optimization of the PEGDA M_n 700 concentration

To study the effect of PEGDA M_n 700 concentration on the monolith performance, lower and higher concentrations were tried using the optimum concentration of Tween®

60. As shown in **Table 3**, increasing the concentration of PEGDA M_n 700 led to a marked improvement in column efficiency up to 30wt% monomers. However, the increased efficiency was observed at expenses of column permeability. 35 and 39 wt% PEGDA (C15 and C16, respectively) led to non-permeable columns. Column C14, that was prepared using 0.30 g PEGDA M_n 700 (30wt%), 0.30 g Tween® 60 (30wt%), 0.16 g decane (16wt%), 0.12 g decanol (12wt%) and 0.12 g dodecanol (12wt%) gave the highest efficiency. The highest number of theoretical plates was achieved for sulfaquinoxaline (60,000 plates/m; k'=7.2) using a mobile phase of ACN/water (30:70, v/v) at 1 μL.min^{-1}. These results show that Tween® 60 could be a better alternative to Tween® 40 in the proposed universal porogenic mixture. The chromatographic efficiency achieved using Tween® 60 was almost double the efficiency attained, when Tween® 40 was utilized.

3.4. Mechanical stability, permeability and repeatability

Mechanical stability and permeability are important to ensure that the prepared columns are fully operational stationary phases. The mechanical stability was measured by changing the mobile phase linear velocity from 0.05 to 0.26 cms^{-1} (equivalent to 0.5 to 2.75 μL/min) and recording the back pressure using ACN and water as mobile phases. As shown in **Fig. S5**, a straight line was obtained with both ACN (r=0.9978) and water (r=0.9997), which indicated that the column was mechanically stable to aqueous and organic solvents. The permeability was also measured and found to be 2.29×10^{-15} m^2 using water as a mobile phase.

The column repeatability was assessed by calculating the percent relative standard deviation (%RSD) of the retention times of five benzoic acids derivatives using a mobile phase consisting of 0.1% formic acid in ACN (pH=3.0)/0.1% formic acid in water (pH=3.0) (20:80, v/v) at 2 μL.min^{-1} in the isocratic mode. The run-to-run and column-to-column repeatability showed a %RSD less than 1.01 and 2.62, respectively. More than 200 injections were performed on the test columns without observable changes in performance. The results showed that the prepared monoliths were repeatable and stable enough for routine analysis.

3.5. Applications in pharmaceutical separation

To test the applicability of these monoliths for the separation of pharmaceutically relevant compounds, the separation of two mixtures of pharmaceutically active ingredients were investigated. The first mixture represents a group of commercially available sulfa-drugs that are commonly used as antimicrobial agents. As shown in **Fig. 5**, baseline separation was achieved in less than 12 min using a mobile phase consisting of ACN/water (20:80, v/v) at 2 μLmin^{-1} in the isocratic mode. The second mixture consisted of five different nonsteroidal anti-inflammatory drugs; aspirin, ibuprofen, phenylbutazone, ketoprofen and naproxen. A run time of 15 min was required to fully separate the five analytes (**Fig. 6**) using an isocratic mobile phase consisting of ACN/Water (30:80, v/v) at 2 μLmin^{-1}. Increasing the %ACN in the mobile phase was associated with a corresponding decrease in retention of all analytes of both mixtures which is a typical behaviour of reversed phase mode [27].

4. Conclusion

A highly efficient PEGDA M_n 700 monolith was prepared using Tween® 60 as a co-porogenic agent. A mixture of decane, decanol, and dodecanol was used as macroporogens and the polymerisation was conducted under thermal conditions. Compared to other polysorbates, Tween® 60 offered higher efficiency and methylene selectivity. The slightly lower hydrophilic-lipophilic balance (HLB) of Tween® 60 may explain the observed enhancement in performance. However, the narrow HLB range in the Tween® series and the differences in functionality and degrees of substitution make it difficult to make a solid conclusion about how HLB of surface active agents can affect the performance. This work shed the light on the potential use of surfactants in monolith recipes. Exploring other types of non-ionic surfactants and the effect of HLB is the target of our future work.

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CRediT author statement

Fotouh R. Mansour: Conceptualization, Formal Analysis, Methodology, Data curation, Writing - original draft. **Christopher T. Desire:** Formal Analysis, Methodology, Writing - review and editing. **Emily F. Hilder:** Conceptualization, Supervision, Funding

acquisition, Writing - review and editing. **R. Dario Arrua:** Conceptualization, Formal Analysis, Supervision, Writing - review and editing.

Declaration of competing interest

The authors declare no competing financial interest.

5. References

[1] S. Hjertén, J.-L. Liao, R. Zhang, High-performance liquid chromatography on continuous polymer beds, J. Chromatogr. A. 473 (1989) 273–275. doi:10.1016/S0021-9673(00)91309-8.

[2] F. Svec, J.M.J. Frechet, Continuous rods of macroporous polymer as high-performance liquid chromatography separation media, Anal. Chem. 64 (1992) 820–822. doi:10.1021/ac00031a022.

[3] R.D. Arrua, T.J. Causon, E.F. Hilder, Recent developments and future possibilities for polymer monoliths in separation science., Analyst. 137 (2012) 5179–89. doi:10.1039/c2an35804b.

[4] F. Svec, Y. Lv, Advances and recent trends in the field of monolithic columns for chromatography., Anal. Chem. 87 (2015) 250–73. doi:10.1021/ac504059c.

[5] P. Jandera, Advances in the development of organic polymer monolithic columns and their applications in food analysis--a review., J. Chromatogr. A. 1313 (2013) 37–53. doi:10.1016/j.chroma.2013.08.010.

[6] T. Zhu, K.H. Row, Preparation and applications of hybrid organic-inorganic monoliths: a review., J. Sep. Sci. 35 (2012) 1294–302. doi:10.1002/jssc.201200084.

[7] J. Urban, Current trends in the development of porous polymer monoliths for the separation of small molecules., J. Sep. Sci. 39 (2016) 51–68. doi:10.1002/jssc.201501011.

[8] F.R. Mansour, S. Waheed, B. Paull, F. Maya, Porogens and porogen selection in the preparation of porous polymer monoliths, J. Sep. Sci. 43 (2020) 56–69. doi:10.1002/jssc.201900876.

[9] P. Aggarwal, H.D. Tolley, M.L. Lee, Monolithic bed structure for capillary liquid chromatography, J. Chromatogr. A. 1219 (2012) 1–14.

[10] A.I. Cooper, A.B. Holmes, Synthesis of molded monolithic porous polymers using supercritical carbon dioxide as the porogenic solvent, Adv. Mater. 11

(1999) 1270–1274. http://cat.inist.fr/?aModele=afficheN&cpsidt=10277578 (accessed May 3, 2016).

[11] K.-F. Du, D. Yang, Y. Sun, Fabrication of high-permeability and high-capacity monolith for protein chromatography., J. Chromatogr. A. 1163 (2007) 212–8. doi:10.1016/j.chroma.2007.06.030.

[12] L. Bai, J. Wang, H. Zhang, S. Liu, J. Qin, H. Liu, Ionic liquid as porogen in the preparation of a polymer-based monolith for the separation of protein by high performance liquid chromatography, Anal. Methods. 7 (2015) 607–613. doi:10.1039/C4AY02200A.

[13] C.T. Desire, R.D. Arrua, M. Talebi, N.A. Lacher, E.F. Hilder, Poly(ethylene glycol)-based monolithic capillary columns for hydrophobic interaction chromatography of immunoglobulin G subclasses and variants., J. Sep. Sci. 36 (2013) 2782–92. doi:10.1002/jssc.201300558.

[14] P. Aggarwal, J.S. Lawson, H.D. Tolley, M.L. Lee, High efficiency polyethylene glycol diacrylate monoliths for reversed-phase capillary liquid chromatography of small molecules, J. Chromatogr. A. 1364 (2014) 96–106. doi:http://dx.doi.org/10.1016/j.chroma.2014.08.056.

[15] J. Chamieh, Y. Zimmermann, A. Boos, A. Hagège, Preparation and characterisation of silica monoliths using a triblock copolymer (F68) as porogen, J. Colloid Interface Sci. 340 (2009) 225–229. doi:10.1016/j.jcis.2009.09.004.

[16] Y. Li, H.D. Tolley, M.L. Lee, Preparation of monoliths from single crosslinking monomers for reversed-phase capillary chromatography of small molecules, J. Chromatogr. A. 1218 (2011) 1399–1408. doi:10.1016/j.chroma.2011.01.028.

[17] K. Nakanishi, T. Nagakane, N. Soga, Designing Double Pore Structure in Alkoxy-Derived Silica Incorporated with Nonionic Surfactant, J. Porous Mater. 5 (1998) 103–110. doi:10.1023/A:1009633102016.

[18] Y. Li, H.D. Tolley, M.L. Lee, Preparation of polymer monoliths that exhibit size exclusion properties for proteins and peptides., Anal. Chem. 81 (2009) 4406–13. doi:10.1021/ac900364d.

[19] O. Kornyšova, A. Maruška, P.K. Owens, M. Erickson, Non-particulate (continuous bed or monolithic) acrylate-based capillary columns for reversed-phase liquid chromatography and electrochromatography, J. Chromatogr. A. 1071 (2005) 171–178. doi:10.1016/j.chroma.2004.10.078.

[20] Y. Li, H.D. Tolley, M.L. Lee, Size-exclusion separation of proteins using a biocompatible polymeric monolithic capillary column with mesoporosity, J. Chromatogr. A. 1217 (2010) 8181–8185. doi:10.1016/j.chroma.2010.10.067.

[21] F.R. Mansour, R.D. Arrua, C.T. Desire, E.F. Hilder, Non-ionic Surface Active Agents as Additives toward a Universal Porogen System for Porous Polymer Monoliths, Anal. Chem. 93 (2021) 2802–2810.

doi:10.1021/acs.analchem.0c03889.

[22] S. Brunauer, P.H. Emmett, E. Teller, Adsorption of gases in multimolecular layers, J. Am. Chem. Soc. 60 (1938) 309–319. doi:10.1021/ja01269a023.

[23] J. Liu, X. Sun, M.L.M. Lee, Adsorption-resistant acrylic copolymer for prototyping of microfluidic devices for proteins and peptides, Anal. Chem. 79 (2007) 1926–31. doi:10.1021/ac0617621.

[24] T. Rohr, E.F. Hilder, J.J. Donovan, F. Svec, J.M. Frechet, J.M.J. Fréchet, Photografting and the control of surface chemistry in three-dimensional porous polymer monoliths, Macromolecules. 36 (2003) 1677–1684. doi:10.1021/ma021351w.

[25] A.J.P. Martin, Some theoretical aspects of partition chromatography, Biochem. Soc. Symp. 3 (1950) 4 – 20.

[26] D. Moravcová, P. Jandera, J. Urban, J. Planeta, Characterization of polymer monolithic stationary phases for capillary HPLC, J. Sep. Sci. 26 (2003) 1005–1016. doi:10.1002/jssc.200301498.

[27] F.R. Mansour, N.D. Danielson, Multimodal liquid chromatography of small molecules, Anal. Methods. 5 (2013) 4955–4972. doi:10.1039/c3ay40302e.

Figure Captions:

Fig. 1. Chemical structures of the studied polysorbate surfactants. In all types, w+x+y+z=20.

Fig. 2. SEM images of the monoliths C0−C5. Monolith compositions and characteristics are summarized in **Table 1**.

Fig. 3. Chromatographic separation of seven different benzoic acid derivatives. Conditions: mobile phase: 0.1% formic acid in ACN(pH=3.0)/0.1% formic acid in water(pH=3.0) (10:90, v/v), injection volume: 50nL, flow rate: 2 μL.min^{-1}, column: C3, temperature: 30°C, Detector: UV at 214 nm. Peak identification in order of elution: **1**: 2,3-dihydroxybenzoic acid (2,3-DHBA), **2**: benzoic acid (BA), **3**: 3-hydroxybenzoic acid (3-HBA), **4**: 4-hydroxybenzoic acid (4-HBA), **5**: 2,4-dihydroxybenzoic acid (2,4-DHBA), **6**: 3,4,5-trihydroxybenzoic acid (3,4,5-THBA) and **7**: 3,5-dihydroxybenzoic acid (3,5-DHBA)

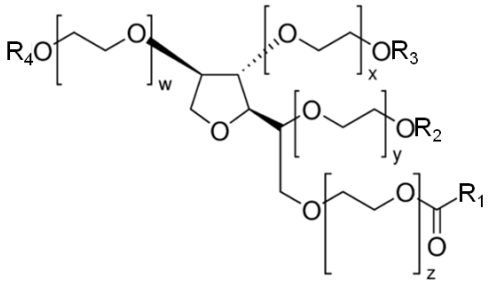
Fig. 4. SEM images of the monoliths C6−C10. Monolith compositions and characteristics are summarized in **Table 2**.

Fig. 5. Chromatographic separation of five different sulfa drugs. Conditions: column: C14 (10 cm × 150 μm ID column), isocratic elution using ACN/water (30:70, v/v); injection volume, 50nL; flow rate, 2 μL.min^{-1}; column temperature, 30ºC, Detector: UV at 214 nm. Peak identifications in order of elution: **1** sulfamerazine, **2** sulfamethizole, **3** sulfamethoxazole, **4** sulfadimethoxine, **5** sulfaquinoxaline.

Fig. 6. Chromatographic separation of five different non-steroidal anti-inflammatory drugs. Conditions: column: C14 (10 cm × 150 μm ID column), isocratic elution using

ACN/water (20:80, v/v); other conditions as in **Fig. 5**. Peak identification in order of elution: **1** aspirin, **2** phenylbutazone, **3** ketoprofen, **4** ibuprofen, **5** naproxen.

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	R_1	R_2	R_3	R_4
Tween 20	$CH_3(CH_2)_9CH_2$—	H	H	H
Tween 40	$CH_3(CH_2)_{13}CH_2$—	H	H	H
Tween 60	$CH_3(CH_2)_{15}CH_2$—	H	H	H
Tween 80	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$—	H	H	H
Tween 85	$CH_3(CH_2)_7CH{=}CH(CH_2)_7$—	H	$=R_1$	$=R_1$

454

455

456 **Fig. 1.** Chemical structures of the studied polysorbate surfactants. In all types,

457 w+x+y+z=20.

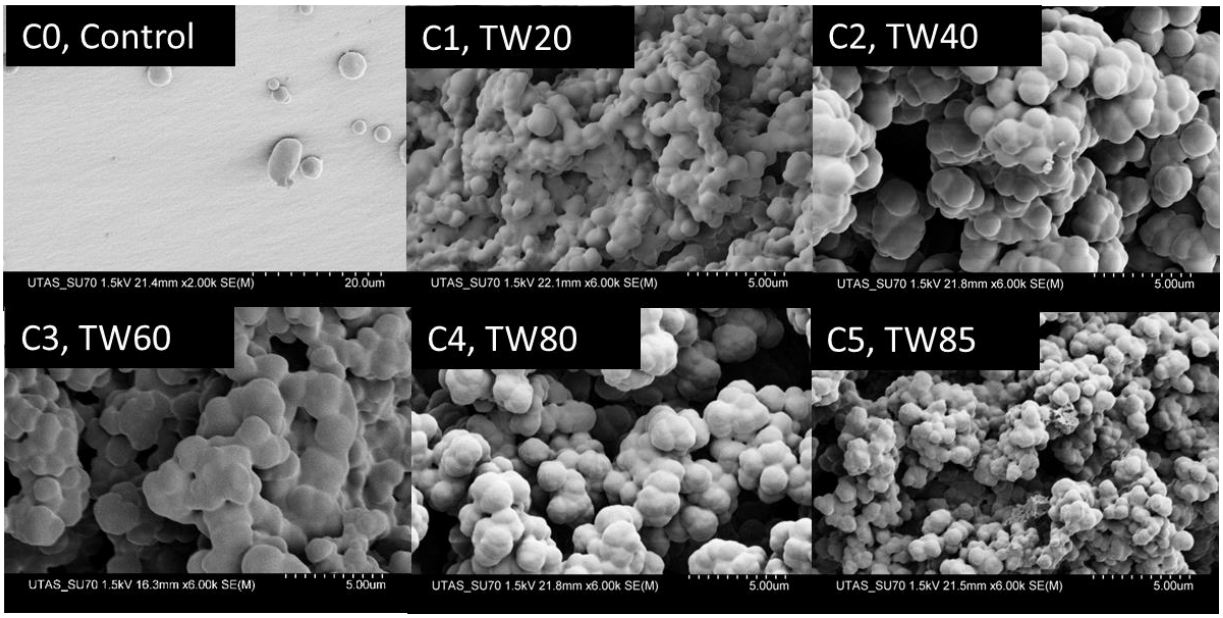


Fig. 2. SEM images of the monoliths C0−C5. Monolith compositions and characteristics are summarized in **Table 1**.

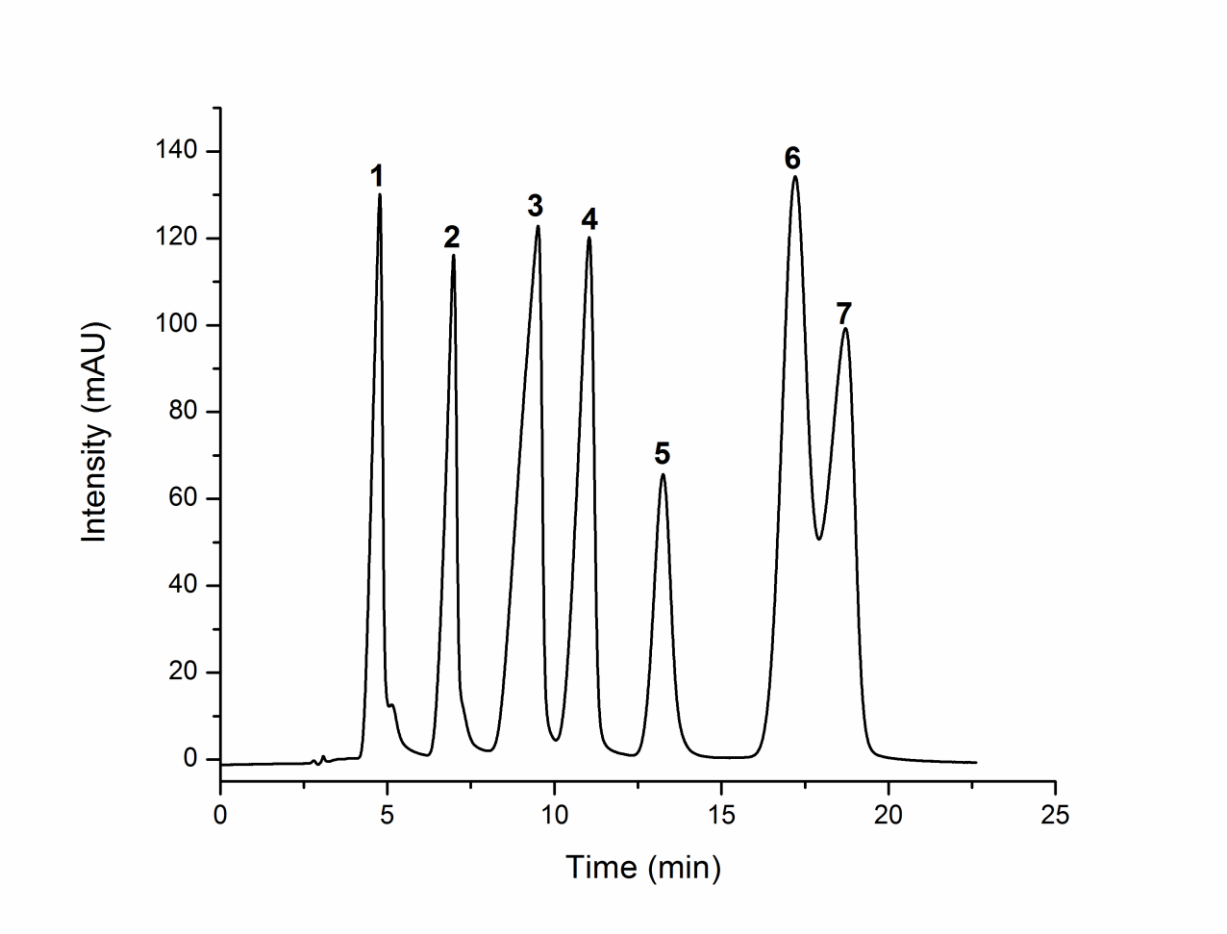


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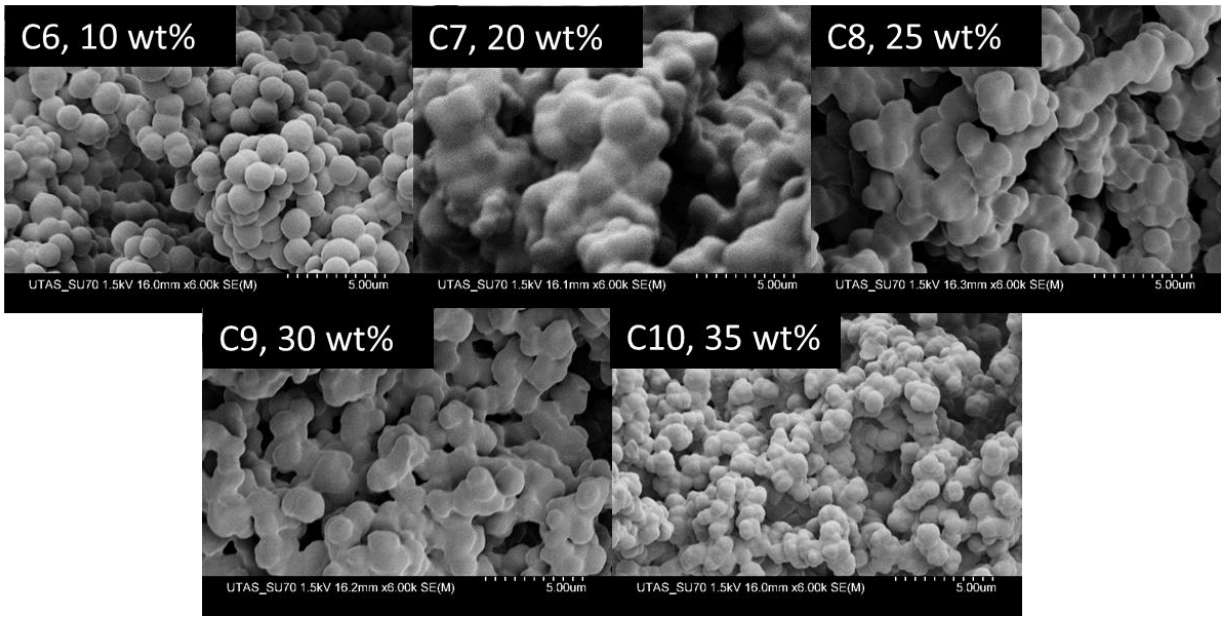


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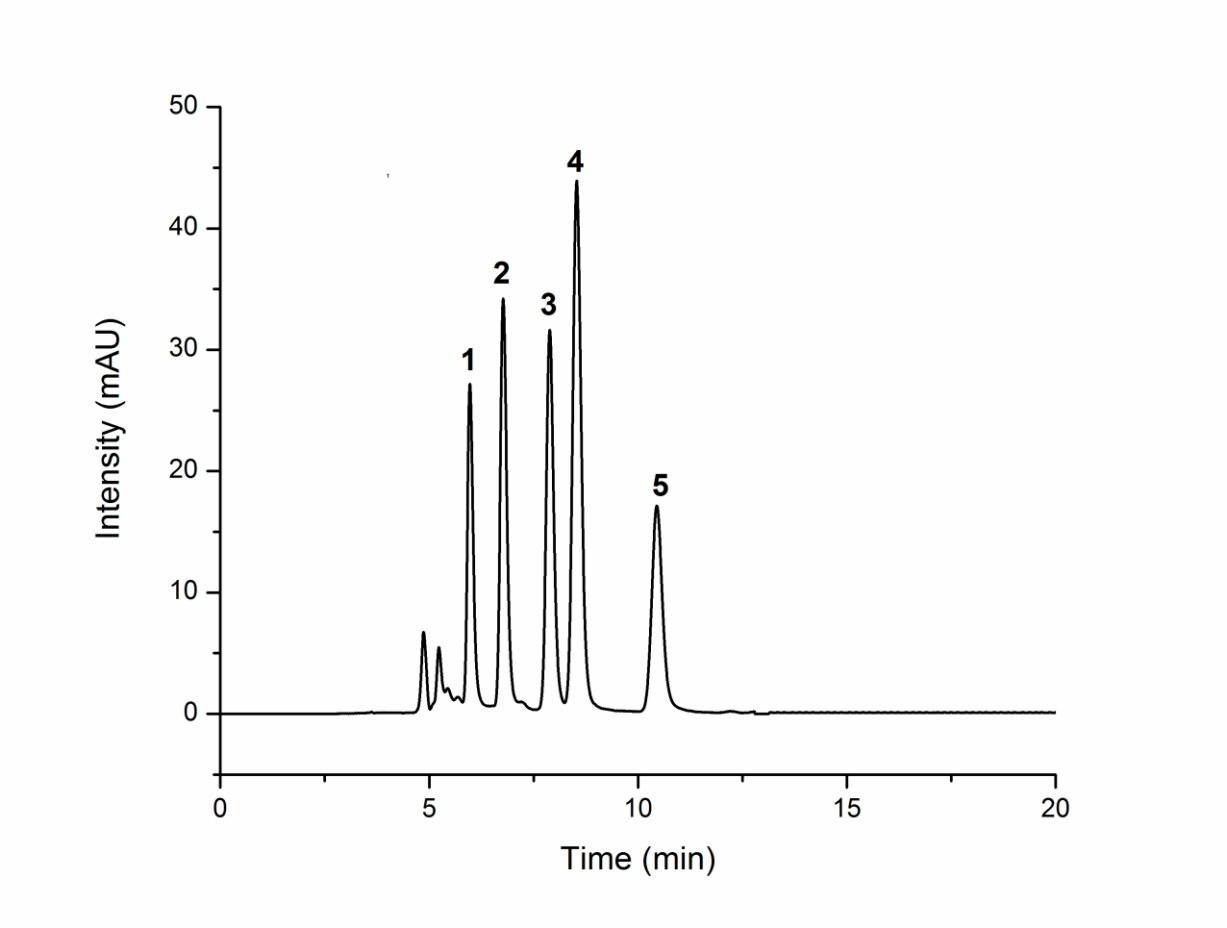


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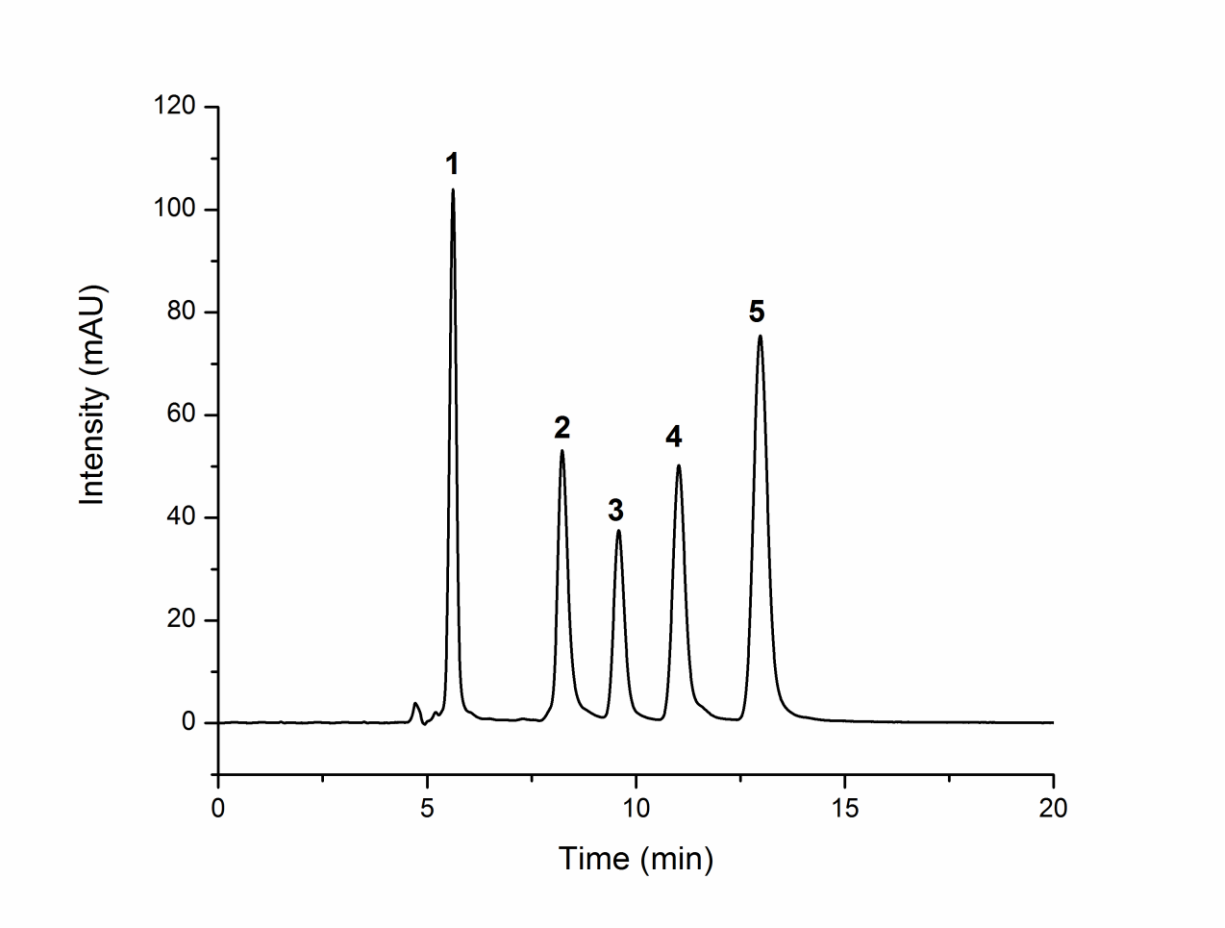


Fig. 6. Chromatographic separation of five different non-steroidal anti-inflammatory drugs. Conditions: column: C14 (10 cm × 150 μm ID column), isocratic elution using ACN/water (20:80, v/v); other conditions as in **Fig. 5**. Peak identification in order of elution: **1** aspirin, **2** phenylbutazone, **3** ketoprofen, **4** ibuprofen, **5** naproxen.

Table 1. Characteristics of monolithic capillaries prepared from different polysorbate surfactants. Each capillary was prepared using 0.2 g PEGDA M_n 700 (20wt%), 0.30 g Tween® (30wt%), 0.16 g decane (20wt%), 0.12 g decanol (15wt%) and 0.12 g dodecanol (15wt%)

Column	SAA in porogen	BET surface area (m^2/g)	Efficiency (Plate/m)	Permeability ($K \times 10^{-14}$ m^2)	Methylene selectivity
C0	–	0.25	NA	NA	NA
C1	TW20	1.02	12300	1.50	1.48
C2	TW40	1.24	14280	1.54	1.46
C3	TW60	1.97	23960	0.96	1.52
C4	TW80	2.73	NA	NA	NA
C5	TW85	3.09	5040	2.09	1.57

Table 2. Characteristics of monolithic capillaries prepared from different concentrations of Tween® 60

Column	wt%TW60	BET surface area (m^2/g)	Efficiency (Plate/m)	Permeability ($K \times 10^{-14}$ m^2)
C6	10	0.31	260	2.89
C7	20	1.24	7140	1.54
C8	25	1.73	14500	1.25
C9	30	1.97	23960	0.96
C10	35	2.19	NA	NA

Table 3. Characteristics of monolithic capillaries prepared from different concentrations of PEGDA M_n 700

Column	wt% PEGDA *Mn* 700	BET surface area (m^2/g)	Efficiency (Plate/m)	Permeability ($K \times 10^{-14}$ m^2)
C11	10	0.57	980	8.33
C12	18	0.76	13,500	4.12
C13	24	2.23	29,000	0.71
C14	30	2.46	46,000	0.23
C15	35	3.12	NA	NA
C16	39	3.39	NA	NA