Zarah Walsh[1]
Pavel A. Levkin[2]*
Vijay Jain[2]
Brett Paull[1]
Frantisek Svec[2]
Mirek Macka[1]

[1]Irish Separation Science Cluster, National Centre for Sensor Research and School of Chemical Sciences, Dublin City University, Glasnevin, Dublin, Ireland
[2]The Molecular Foundry, E.O. Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Received September 28, 2009
Revised October 29, 2009
Accepted October 30, 2009

Research Article

Visible light initiated polymerization of styrenic monolithic stationary phases using 470 nm light emitting diode arrays

Poly(styrene-*co*-divinylbenzene) monolithic stationary phases have been synthesized for the first time by photoinitiated polymerization. An initiator composed of (+)-(*S*)-camphorquinone/ethyl-4-dimethylaminobenzoate/*N*-methoxy-4-phenylpyridinium tetrafluoroborate was activated using a 470 nm light emitting diode array as the light source. Spatially controlled polymerization of styrenic monoliths has been achieved within specific sections of a 100 μm id polytetrafluoroethylene-coated fused-silica capillary using simple photo masking. The sharpness of the edges was confirmed by optical microscopy, while SEM was used to verify a typical porous, globular morphology. Flow resistance data were used to assess the permeability of the monoliths and they were found to have good flow through properties with a flow resistance of 0.725 MPa/cm at 1 μL/min (water, 20°C). Conductivity profiling along the length of the capillary was used to assess their lateral homogeneity. Monoliths which were axially rotated during polymerization were found to be homogeneous along the whole length of the capillary. The monolithic stationary phases were applied to the RP gradient separation of a mixture of proteins. Column fabrication showed excellent reproducibility with the retention factor (*k*) having a RSD value of 2.6% for the batch and less than 1.73% on individual columns.

Keywords: LED / Monolith / Photoinitiated polymerization / Styrene / Visible light
DOI 10.1002/jssc.200900624

1 Introduction

Poly(styrene-*co*-divinylbenzene) stationary phases were first introduced by Horváth *et al.* [1, 2] when they copolymerized styrene and divinylbenzene (DVB) on the surface of pellicular glass beads for the separation of nucleotides. This was followed by Maa and Horváth in 1988 [2, 3] by the synthesis of non-porous particles made entirely of poly(styrene-*co*-divinylbenzene), which were used for the separation of proteins. Today, commercially available porous poly(styrene-*co*-divinylbenzene) beads are largely used in size exclusion chromatography and adsorption processes. A recent development in polymer stationary phases was the introduction of monolithic porous polymers in 1992 [4]. The first poly(styrene-*co*-divinylbenzene) monoliths were prepared using thermally initiated polymerization to achieve a rigid, macroporous, interconnected polymer structure [4, 5].

Photo-initiated polymerization is extremely useful for the synthesis of spatially controlled monolithic stationary phases in capillary; however, it is most commonly carried out using UV light [6, 7]. The use of UV light sources limits both the pre-polymer solution itself, and the material within which the monolith can be polymerized, to those which are UV transparent. As styrene and DVB absorb strongly in the UV region of the spectrum the state of the art for the synthesis of poly(styrene-*co*-divinylbenzene) monoliths remains thermally initiated polymerization. A further issue arising with photo-initiated polymerization relates to the fact that the wavelength of maximum absorbance of the initiator, porogens and monomers can overlap and each of these compounds can compete for photons from the light source which in turn makes efficient polymerization difficult.

Although difficult, the preparation of poly(styrene-*co*-divinylbenzene) particles has been reported in the literature using a conventional UV light source utilizing both standard free radical and so-called reversible addition-fragmentation chain transfer polymerization mechanisms [8–11]. For example, Limé and Irgum [8] used standard free radical polymerization with azobisisobutyronitrile as the photo-

Correspondence: Dr. Mirek Macka, School of Chemical Sciences, Dublin City University, Glasnevin, Dublin 9, Ireland
E-mail: mirek.macka@gmail.com
Fax: +353-1-700-5503

Abbreviations: **CQ,** (+)-(*S*)-camphorquinone; **DVB,** divinylbenzene; **EDAB,** ethyl-4-dimethylaminobenzoate; **LED,** light emitting diode; **MPPB,** *N*-methoxy-4-phenylpyridinium tetrafluoroborate; **PTFE,** polytetrafluoroethylene

*Current address: Dr. Pavel A. Levkin, Forschungszentrum Karlsruhe and Heidelberg University, Germany

initiator to synthesize poly(styrene-*co*-divinylbenzene) particles. Azobisisobutyronitrile was used as it absorbs at 365 nm, which is just outside the absorbance band of both styrene and DVB. However, Limé and Irgum reported excessively long and impractical polymerization times of 24–163 h.

To our best knowledge, the photoinitiated polymerization of styrene and DVB to form monolithic stationary phases has not been demonstrated within fused-silica capillary columns or in channels within micro-fluidic chips. Therefore, the aim of this work was to photoinitiate and spatially control the preparation of poly(styrene-*co*-divinylbenzene) monoliths within poly(tetrafluoroethylene) (PTFE)-coated fused-silica capillaries, using an array of 470 nm light emitting diodes (LEDs) as the initiating light source.

2 Materials and methods

2.1 Reagents

1-Decanol, 1-propanol, 3-(trimethoxysilyl)propyl methacrylate, cytochrome *c*, DVB, ethyl-4-dimethylamino benzoate (EDAB), formic acid, HPLC grade acetonitrile (ACN), myoglobin, *N*-methoxy-4-phenylpyridinium tetrafluoroborate (MPPB), ovalbumin, ribonuclease A, CQ and styrene were all purchased from Sigma-Aldrich Chemical (St. Louis, MO, USA). All chemicals were used as received with the exception of monomers which were purified before use by passing over a bed of basic aluminium. Water was obtained from a Milli-Q Ultrapure water filtration system from Millipore (Billerica, MA, USA).

2.2 Materials

A 470 nm LED array (RLT-MIL470-12B-30) purchased from Roithner LASER Technik (Vienna, Austria) consisted of 12 LEDs emitting at 470 nm with 20 mW of power each. PTFE-coated fused-silica capillaries, with an internal diameter of 100 μm, were obtained from Polymicro Technologies (Phoenix, AZ, USA) and their internal walls were silanized prior to use with 3-(trimethoxysilyl)propyl methacrylate using a procedure described elsewhere [12].

2.3 Preparation of poly(styrene-*co*-divinylbenzene) monoliths

0.64 mg (3.86 μmol) CQ, 3.2 mg (16.57 μmol) EDAB and 3.2 mg (17.18 μmol) MPPB were weighed into a vial and dissolved in 130 μL of a mixture of ACN, 1-propanol and 1-decanol in a ratio 18:36:46 v/v/v. The vial was shaken to dissolve all components of the initiator system. Thirty-five μL (305 μmol) styrene and 35 μL (246 μmol) DVB were then added and the solution was sonicated for 20–30 min to ensure complete mixing of the solutions and to remove dissolved oxygen which can interfere with the polymerization process. Capillaries were filled by capillary action. The ends of the capillaries were sealed with rubber septa and black vinyl tape. A combination of black vinyl tape and rubber septa were used to mask the capillaries for polymerization as rubber septa are very effective in stopping evanescent wave polymerization and black vinyl tape was necessary as the rubber septa are not long enough to mask the entire length of the capillary where polymerization should not occur. Capillaries were placed beneath the LED array at a distance of 1 cm and irradiated for a period of 2 h with rotation at approximately 34 rpm. The capillary was rotated by using a small motor modified by gluing a standard capillary connector onto its rotating central shaft. The capillary was attached to the connector so the capillary was secure and positioned horizontally at all times while being rotated during polymerization. This set-up was to ensure that all parts of the capillary receive equal irradiation from the light source to achieve homogeneous polymer formation.

Control experiments were carried out to determine the time necessary to initiate polymerization under ambient light conditions. The polymerization mixture was placed in a clear vial, labeled and placed in the bright lab without specific irradiation, after 4 h polymer formation was observed in the bottom of the vial and within 24 h the solution was fully polymerized. As a precaution all solutions for polymerization were then made up in amber vials and polymerization was carried out in the dark to ensure that the polymerization mixture was not exposed to any ambient light which may interfere with the LED initiated polymerization.

2.4 Characterization

The decomposition of the dye sensitizer in the presence of different co-initiators was monitored using a Cary 50 UV–Vis spectrometer (Varian, Palo Alto, CA, USA).

Flow resistance measurements and the separation of proteins in monolithic capillaries were carried out using an Ultimate 3000 nano-HPLC from Dionex (Sunnyvale, CA, USA). Analyses were carried out at a flow rate of 1 μL/min, with an injected sample volume of 50 nL and a detection wavelength of 210 nm. The gradient elution profile consisted of 0.1 vol% formic acid in water (mobile phase A) for 1 min, 10 min to go from 0–60 vol% mobile phase B in A (0.1 vol% formic acid in ACN) in 10 min, 5 min hold, return to A in 5 min followed by 5 min conditioning before the next injection. Each separation was run three times on each column.

Optical and scanning electron micrographs of capillaries were taken with a TE200 optical microscope from Nikon (Tokyo, Japan) and an Ultra-55 analytical scanning electron microscope from Carl Zeiss (Jena, Germany), respectively. After synthesis the porogens were removed

from the monolith by flushing with ACN and air using a hand-syringe. This approach emphasizes the contrast between the empty and filled parts of the capillary in optical microscopy.

A Tracedec® capacitively coupled contactless conductivity detector with a 375 μm sensor head from Istech (Vienna, Austria) was used for lateral conductivity profiling. A low-pressure infusion pump (KD Scientific, Holliston, MA, USA) with a 250 μL syringe (Hamilton, Bonaduz, Switzerland) was used to provide a constant flow of water through the capillary during conductivity profiling. An on-column capacitively coupled contactless conductivity detector cell was placed on the capillary filled with water and moved down the length of the filled capillary at 1-mm increments, the conductivity value was recorded at each interval. While the stationary phase bears no charge, voids in the columns result in decrease in conductivity.

3 Results and discussion

Figure 1 shows the absorbance spectra of a 0.51 mol/L solution of styrene and a 0.41 mol/L solution of DVB in an ACN, 1-propanol and 1-decanol mixture, overlaid with the emission spectra of 255, 365 and 470 nm LEDs. The concentration of the monomers is approximately 20% of the concentration in a typical pre-polymer solution used for monolith production.

Figure 1 shows that the monomers absorb very strongly up to 330 nm, fully absorbing the emission of the 254 nm LED, and showing substantial overlap with the emission peak of the 365 nm LED when present at concentrations required for monolith fabrication, which is five times higher than the concentrations in Fig. 1. There is, therefore, competition for photons between the monomers and any initiator which also absorbs at these wavelengths. This competition results in a subsequent reduction in initiation efficiency. In contrast, the emission of the 470 nm LED is sufficiently spectrally resolved from the absorbance of the monomers and therefore represents a suitable wavelength at which to initiate the polymerization, provided a suitably efficient initiating system active at 470 nm can be exploited.

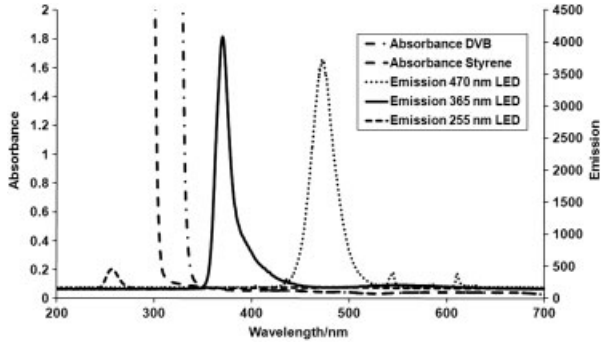


Figure 1. Absorbance spectra of styrene (0.51 mol/L, 20% of concentration in pre-polymer solution), divinylbenzene (0.41 mol/L, 20% of concentration in pre-polymer solution) and emission spectra of 254, 365 and 470 nm LEDs. Monomers are dissolved in a solution of ACN/1-propanol/1-decanol.

It is known that CQ is an excellent photosensitizer for the initiation of free radical polymerization *via* photo-induced electron transfer when irradiated at 470 nm [13–18]. Typically, EDAB is used as a co-initiator with CQ to complete the electron transfer/radical generation process. This combination was therefore explored as the initiating system.

3.1 Optimization of the polymerization mixture

THF and 1-decanol are commonly used as the micro-porogen and macro-porogen, respectively, for the preparation of poly(styrene-*co*-divinylbenzene) monoliths in thermally initiated free radical polymerizations [4, 19, 20]. However, Oxman *et al.* [17] have found that CQ in the presence of THF did not initiate the conversion of monomer to polymer. A mixture of ACN/1-propanol/1-decanol was therefore used as the porogenic solvent.

A series of control experiments were run using constant concentrations of porogens and monomers while varying the concentration of the initiator. We found that 1 wt% CQ and 5 wt% EDAB (% of the total weight of the monomers) afforded the highest conversion to polymer at a given time. The optimum pre-polymer solution was then used for the preparation of monoliths in capillaries. GC analysis of the residual liquid in the capillary revealed that even after 6 h of polymerization, both styrene and DVB were still present, suggesting an incomplete conversion. Low resistance to flow indicated a very porous monolith, which is a further indication of a poor conversion.

It was shown in a previous work on the visible light initiated polymerization of methacrylates [21] that MPPB significantly increased the rate of polymerization. Thus, we investigated different concentrations of this salt in the pre-polymer solution and found that keeping the ratio of EDAB and MPPB equal led to a high conversion, even though the polymerization time was reduced from 6 to 2 h after the addition of 5 wt% MPPB.

To ensure that the same response would not have been obtained by simply increasing the concentration of either of the co-initiators, control experiments were conducted with 1 wt% CQ/10 wt% EDAB and 1 wt% CQ/10 wt% MPPB as the initiators. These systems did not afford capillaries completely filled with monolith, thus confirming that it is the combination of all the components that is responsible for the increase in the rate of polymerization.

Figure 2 shows the decomposition of the initiator; the lines marked CQ are the spectra of the original 1% CQ in the porogenic solvent and after 120 min irradiation. The same system with the addition of 5 wt% EDAB to the mixture exhibits red shifted absorbance (marked CQE) but no increase in the absorbance at 470 nm. This means that no additional electrons are transferred as no additional light is absorbed. Addition of 5 wt% MPPB (marked CQEM) to

the solution causes an increase in the absorbance at 470 nm indicating that more electrons transferred to the co-initiator generate more free radicals and therefore increase the rate of the reaction. As can be seen in all spectra the peak at 470 nm disappears after 120 min of irradiation, signaling the end of the polymerization as without any further generation of radicals the chain growth cannot continue and the polymerization is terminated.

3.2 Preparation and characterization of monoliths

Photo-initiated polymerization is a useful tool to control diffusion at the ends of the monolithic stationary phase.

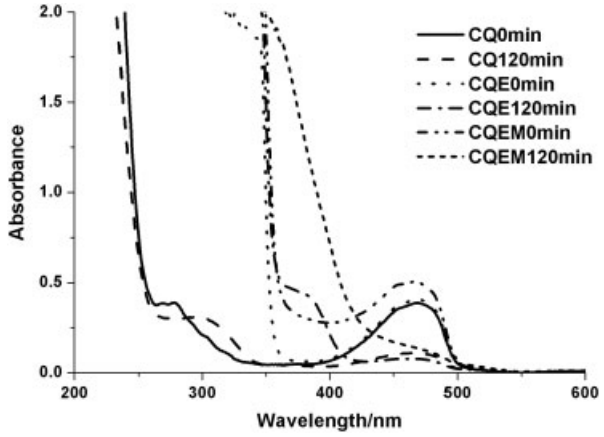


Figure 2. Effect of the addition of components of the initiator complex on the absorbance of the dye sensitizer and the rate of the reaction. The lines represented on the legend by CQ (0 min, 120 min) are the spectra of a solution containing 1 wt% CQ only, the lines represented by CQE (0 min, 120 min) have 5 wt% EDAB added to the original CQ solution and the lines represented by CQEM (0 min, 120 min) have 5 wt% EDAB and 5 wt% MPPB added to the original solution.

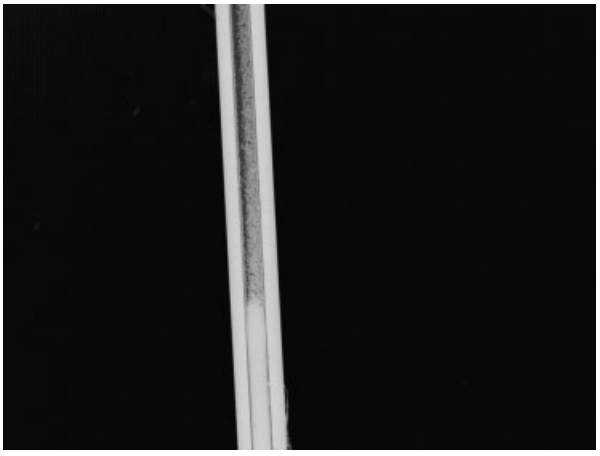


Figure 3. Optical micrograph of the edges of the poly(styrene-*co*-divinylbenzene) monolith within a poly(tetrafluoroethylene) coated fused-silica capillary.

Using a simple photo-mask the location of the monolith within the capillary or channel can be controlled very easily. As PTFE coated fused-silica capillary is an effective light waveguide, a more efficient mask than simply using black vinyl tape must be employed. For this reason, rubber septa were placed at the ends of the capillary to block light from propagating along the length that would lead to polymerization even in the masked parts. Figure 3 shows the edge of the monolith formed within the capillary using the described method. The image confirms that the edges of the polymer monolith in capillary are acceptably sharp.

SEM (Fig. 4) was also used to characterize the monoliths. The images show that the polymerization results in the expected polymer monolith structure, with a well developed globular, macroporous arrangement. The images also confirm that the capillary is completely filled with monolith and that it is firmly attached to the wall.

The pressure drop of a batch of three columns prepared using the optimized conditions was measured using water as the eluent (Fig. 5). Very good repeatability for the new preparation method is demonstrated, with less than 10% deviation between the pressure drop values recorded for each of the three columns.

As the LED array used here for polymerization consisted of two rows of LEDs 1 cm apart there are points of high and low light intensity along the length of the capillary which result in alternating plugs of dense and permeable mono-

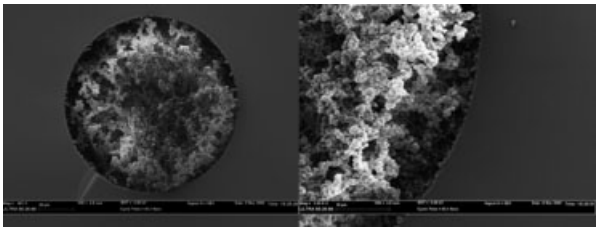


Figure 4. Scanning electron micrograph of a 100 μm id PTFE coated fused-silica capillary filled with a poly(styrene-*co*-divinylbenzene) monolith.

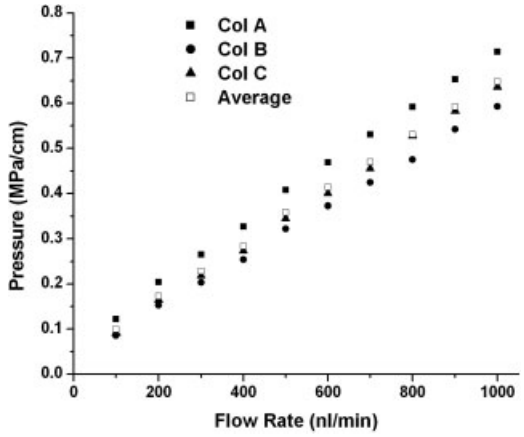
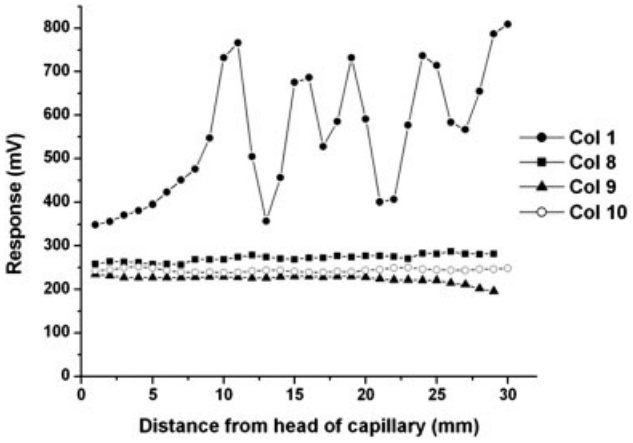


Figure 5. Flow resistance poly(styrene-*co*-divinylbenzene) monoliths in capillary.



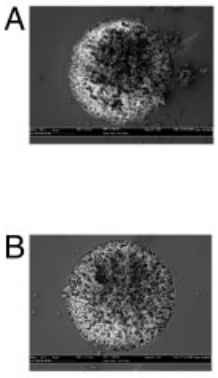


Figure 6. Capacitively coupled contactless conductivity detection profile of four monolithic stationary phases which have been kept stationary (Col 1) or rotated (Col 8, Col 9 and Col 10) during polymerization. Scanning electron micrographs of poly-(styrene-*co*-divinylbenzene) monoliths with approximately 60% pore volume which have been left stationary (A) and rotated (B), during polymerization under the optimum conditions are shown on the right hand side.

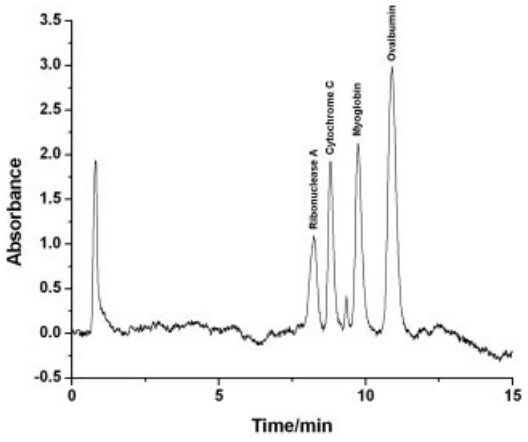


Figure 7. Example of a separation of a mixture of proteins using poly(styrene-*co*-divinylbenzene) monolith. Peaks (order of elution) are ribonuclease A, cytochrome c, myoglobin and ovalbumin. Separation was carried out at a flow rate of 1 μL/min, gradient of 0–60% 0.1 vol% formic acid in ACN in 10 min; UV detection was at 210 nm.

lith. Axial rotation of the capillary during polymerization was therefore found to be very important to the preparation of homogeneous monoliths. As the capillary filled with the pre-polymer solution is constantly rotated during the polymerization step, these dense/permeable bands disperse and the monolith is more homogeneous along its length. Conductivity profiling along the length of the column was used to determine the homogeneity of the poly(styrene-*co*-divinylbenzene) monoliths. This method was described by Gillespie *et al.* [22] as an accurate method of evaluating the homogeneity of a monolith in a non-destructive manner.

The homogeneity was recorded for monoliths prepared using two different methods. Figure 6 shows the scanning electron micrographs of these monoliths, one of which has been rotated during polymerization (A) and another which has been kept stationary (B). No difference between these two images can be observed visually. In contrast, a conductivity longitudinal profile monitored for four monoliths with three rotated and one stationary during polymerization are clearly different, with the former showing excellent longitudinal homogeneity of porous structure.

Table 1. Comparison of retention factors (*k*) for three poly(styrene-*co*-divinylbenzene) monoliths in capillary columns with SDs and % RSD

Peak	$k' \pm$SD	% RSD
Ribonuclease A	9.48 ± 0.24	2.53
Cytochrome C	10.14 ± 0.25	2.43
Myoglobin	11.36 ± 0.28	2.46
Ovalbumin	12.90 ± 0.33	2.58

Poly(styrene-*co*-divinylbenzene) stationary phases are suitable for the separation of biomolecules such as proteins and peptides [19, 23–25]. Our monoliths were examined for the separation of a mixture of standard proteins; ribonuclease A, cytochrome c, myoglobin and ovalbumin. Figure 7 shows an example of a baseline separation achieved.

Table 1 shows the average retention factors for each protein run on the three monolithic columns. The relative standard deviation for the retention factors were all $\leq 2.58\%$.

The selectivity for the proteins shown in Fig. 7 is the same as that observed by Levkin *et al.* [19] using poly-(styrene-*co*-divinylbenzene) monoliths synthesized by thermally initiated polymerization in a micro-fluidic chip. This confirms that the monoliths synthesized in this work exhibit a very similar selectivity based on their hydrophobic character. The resolution of ribonuclease A and cytochrome c is 1.07. Cytochrome c/myoglobin and the myoglobin/ovalbumin peaks are resolved even better with an average resolution of 2.11 and 2.56, respectively.

4 Concluding remarks

For the first time poly(styrene-*co*-divinylbenzene) monolithic stationary phases have been synthesized using photo-induced polymerization. Initiation with visible light at 470 nm could be conveniently achieved with an LED array.

The physical and chromatographic characterization of the monoliths prepared in PTFE coated fused-silica capillary verified that this procedure affords monolithic columns of comparable characteristics to those produced by thermally initiated polymerization. This new development will allow more flexibility within the area of photo-induced polymerization of monolithic stationary phases, particularly with regard to the monomers and moulds/housing used.

Z.W. and M.M. acknowledge the Marie Curie Excellence Grants and Funding (MEXT-CT-2004-014361) for financial support of this work. Dublin City University School of Chemical Sciences is acknowledged for a travel grant enabling Z.W. to visit the Lawrence Berkeley National Laboratory. B.P. and M.M. wish to thank Science Foundation Ireland for support for the Irish Separation Science Cluster award (Grant Number 08/SRC/B1412). Support of P.L. by a grant of the National Institutes of Health (GM48364) is gratefully acknowledged. F.S. and the experimental work performed at the Molecular Foundry, Lawrence Berkeley National Laboratory, were supported by the Office of Science, Office of Basic Energy Sciences, U.S. Department of Energy, under Contract No. DE-AC02-05CH11231.

The authors have declared no conflict of interest.

5 References

[1] Horváth, C. G., Preiss, B. A., Lipsky, S. R., *Anal. Chem.* 1967, *39*, 1422–1428.

[2] Huck, C. W., Bonn, G. K., *Chem. Eng. Technol.* 2005, *28*, 1457–1472.

[3] Maa, Y.-F., Horváth, C., *J. Chromatogr.* 1988, *445*, 71–86.

[4] Svec, F., Fréchet, J. M. J., *Anal. Chem.* 1992, *64*, 820–822.

[5] Wang, Q. C., Svec, F., Frechet, J. M. J., *Anal. Chem.* 1993, *65*, 2243–2248.

[6] Abele, S., Nie, F.-Q., Foret, F., Paull, B., Macka, M., *Analyst* 2008, *133*, 864–866.

[7] Viklund, C., Pontén, E., Glad, B., Irgum, K., Horstedt, P., Svec, F., *Chem. Mater.* 1997, *9*, 463–471.

[8] Limé, F., Irgum, K., *Macromolecules* 2007, *40*, 1962–1968.

[9] Paczkowski, J., Kucybala, Z., Scigalski, F., Wrzyszczynski, A., *J. Photochem. Photobiol. A* 2003, *159*, 115–125.

[10] Ran, R., Yu, Y., Wan, T., *J. Appl. Polym. Sci.* 2007, *105*, 398–404.

[11] Ran, R., Wan, T., Gao, T., Gao, J., Chen, Z., *Polym. Int.* 2008, *57*, 28–34.

[12] Krenkova, J., Lacher, N. A., Svec, F., *Anal. Chem.* 2009, *81*, 2004–2012.

[13] Corrales, T., Catalina, F., Peinado, C., Allen, S., *J. Photochem. Photobiol. A* 2003, *159*, 103–114.

[14] Emami, N., Soderholm, K.-J. M., *J. Mater. Sci. Mater. Med.* 2005, *16*, 47–52.

[15] Jakubiak, J., Nie, J., Lindén, L. A., Rabek, J. F., *J. Polym. Sci. A Polym. Chem.* 2000, *38*, 876–886.

[16] Krishnan, V. K., Yamuna, V., *J. Oral Rehabil.* 1998, *25*, 747–751.

[17] Oxman, J. D., Jacobs, D. W., Trom, M. C., Sipani, V., Ficek, B., Scranton, A. B., *J. Polym. Sci.: Part A Polym. Chem.* 2005, *43*, 1747–1756.

[18] Yu, Q., Nauman, S., Santerre, J. P., Zhu, S., *J. Mater. Sci.* 2001, *36*, 3599–3605.

[19] Levkin, P. A., Eeltink, S., Stratton, T. R., Brennen, R., Robotti, K., Yin, H., Killeen, K., Svec, F., Fréchet, J. M. J., *J. Chromatogr. A* 2008, *1200*, 55–61.

[20] Premstaller, A., Oberacher, H., Huber, C. G., *Anal. Chem.* 2000, *72*, 4386–4393.

[21] Walsh, Z., Abele, S., Lawless, B., Heger, D., Klán, P., Breadmore, M. C., Paull, B., Macka, M., *Chem. Commun.* 2008, 6504–6506.

[22] Gillespie, E., Macka, M., Connolly, D., Paull, B., *Analyst* 2006, *131*, 886–888.

[23] Ivanov, A. R., Zang, L., Karger, B. L., *Anal. Chem.* 2003, *75*, 5306–5316.

[24] Premstaller, A., Oberacher, H., Walcher, W., Timperio, A. M., Zolla, L., Chervet, J.-P., Cavusoglu, N., van Dorsselaer, A., Huber, C. G., *Anal. Chem.* 2001, *73*, 2390–2396.

[25] Xie, S., Allington, R. W., Svec, F., Frechet, J. M. J., *J. Chromatogr. A* 2000, *865*, 169–174.

