Fabrication of Bonded Monolithic Porous Layer Open Tubular (monoPLOT) Columns in Wide Bore Capillary by Laminar Flow Thermal Initiation

David A. Collins • Ekaterina P. Nesterenko•Dermot Brabazon• Brett Paull

Abstract A novel scalable procedure for the thermally initiated polymerisation of bonded monolithic porous layers of controlled thickness within open tubular fused silica capillaries (monoPLOT columns) is presented. Porous polymer layers of either polystyrene-divinylbenzene or butyl methacrylate-ethylene dimethacrylate, of variable thickness and morphology were polymerised inside fused silica capillaries utilising combined thermal initiation and laminar flow of the polymerisation mixture. The procedure enables the production through thermal initiation of monoPLOT columns of varying length, internal diameter, user defined morphology and layer thickness for potential use in both liquid and gas chromatography. The morphology and thickness of the bonded polymer layer on the capillary wall is strongly dependent on the laminar flow properties of the polymerisation mixture and the changing shear stress within the fluid across the inner diameter of the open capillary. Owing to the highly controlled rate of polymerisation and its dependence on fluid shear stress at the capillary wall, the procedure was demonstrably scalable, as illustrated by the polymerisation of identical layers within different capillary diameters.

Keywords Laminar flow polymerization, Thermally initiated polymerisation monoPLOT columns

Introduction

Open tubular (OT) columns were initially proposed for gas chromatography (GC) by Golay [1] and, following this pioneering development, OT capillary GC has practically replaced packed-column GC for most analytical applications, with porous layer open tubular (PLOT) columns now well established as a common OT column format [2] and such OT columns are typically produced by immobilising a layer of stationary phase on to the inner wall of the column. Because in many cases, it is not chemically bonded to the column wall, the stability of this adsorbed stationary phase layer ultimately depends on the constant nature of the surface tension forces that hold it to the column wall. As a result, stationary phase layers can gradually or suddenly break up and wash off the column—an effect commonly known as column bleed. This effect can be reduced or eliminated completely if the stationary phase is chemically bonded on to the capillary wall. In the case of porous polymer layers, this can only be achieved via in situ polymerisation.

Porous layer structures can provide high capacity due to their higher surface area when compared with non-porous, wall-coated polymeric coatings (WCOT). However, the commercial techniques used in the formation of all such stationary phases upon the walls of capillary columns are of course highly proprietary. This relative lack of bonded polymeric

PLOT columns for use in GC, and indeed capillary liquid chromatography (LC), is due mainly to the many problems associated with the controllable manufacture of such columns of varying dimensions.

Recently, a technique for the production of monoPLOT columns in capillaries of large internal diameter (ID) was published by Collins et al. [3]. The approach utilisedphotoinitiated in situ polymerisation, however, its application was limited to polymers, and resultant polymer structures, which could be produced by photo-initiation within the ultraviolet (UV) region, and within UV transparent media. Polystyrenebased materials are commonly used as reversed phase and hydrophobic substrates in both LC and GC. However, there are a number of difficulties related to the fabrication of open tubular columns with a polystyrene-divinylbenzene (PSDVB) stationary phase. First, photo-initiated polymerisation within the UV region is not possible due to the strong UV absorbance of styrene itself. In addition, the polyimide coatingoffusedsilicacapillaryofthetypecommonlyusedin GC also absorbs in the UV region, up to approximately 600 nm (see Supplementary Information Figure S1), thus making in situ polymerisation by UV photo-initiation impossible regardless of the polymerisation mixture.

Thermal initiation of polymers, such as PS-DVB, although unrestricted by UV absorbance limitations, is notoriously difficult to control in the production of open tubular or layered structures. Earlier studies [4–8] have demonstrated that the fabrication of thermally initiated PLOT type columns is possible, however, stationary phase layers could only be obtained in capillaries of small ID (typically less than 25 μm) where they were successfully applied to capillary LC [5], but these dimensions are too small for application in GC. Chuang et al. [9] has reported the thermal polymerisation of a polystyrene 'monolithic' layer inside a 75 μm ID capillary, yet the layer thickness achieved was only 300 nm with little or no porosity. It is interesting to note that Shen [10] could obtain porous layer structure by in situ polymerisation of divinylbenzene in a 762 μm ID stainless steel tube, with the layer formed through static polymerisation at 80 ^{0}C for 7 h.

This success can be attributed to the fact that stainless steel has a high thermal conductivity when compared with silica, and the polymerisation mixture at the tube walls would reach polymerisation temperature very quickly, when compared with the centre of the tube. However, having such a thermal gradient in the tube results in a highly non-uniform layer, with low porosity and globule size at the wall, which increases dramatically towards the centre. In this study, it was not possible to control either the pore size, morphology or the thickness of the layer. Overall, these limited successes can be mainly ascribed to the difficulty in controlling the thermal polymerisation process as compared to photo-initiated polymerisation. More often than not, attempts to form polymeric PLOT columns through thermal initiation will result in over polymerisation across the entire section of the capillary [4].

Very little work has been done on laminar flow polymerisation within capillaries, and no prior studies have been reported on the fabrication of porous polymer 'monoPLOT' type columns by either 'flow through', or laminar flow thermal polymerisation. Prior reports on laminar flow polymerisation describe exothermic reactions in large scale reactors [11–13] for the bulk manufacture of polymers, which have limited relevance in regard to polymerisation

within micro-bore capillaries of the type discussed herein. In the above-mentioned preliminary study by the current authors [3], flow through photo-initiated polymerisation was attempted with mixed results. From analysis of results herein, it would appear that within this early study the flow velocities used were excessively high, resulting in turbulent flow, less than ideal phase morphology, and loss of pore structure.

Therefore, within this current study, the development of a highly controlled thermally initiated method for the fabrication of PS-DVB monoPLOT columns in large ID capillary (from 50 to 200 μm) is presented. Precise control over layer thickness and morphology was delivered through the application of laminar flow within the monomer mixture through the capillary during polymerisation.

Experimental

Reagents and Materials

All chemicals used within this study were of reagent or analytical grade purity. Ethylene dimethacrylate (EDMA), butyl methacrylate (BuMA), styrene, divinylbenzene, 4-vinylbenzyl chloride, 1-decanol, toluene, 3methoxysilylpropyl methacrylate, trifluoroacetic acid (TFA) and proteins used for chromatographic evaluation of prepared column (Insulin, Ribonuclease B, Trypsin, Ribonuclease A, Cytochrome C, Myoglobin, Horseradish Peroxidase, Phosphatase B, Carbonic Anhydrase, Concanavalin A) were all purchased from Sigma-Aldrich (Gillingham, UK). The thermal initiator, azobisisobutyronitrile (AIBN), was obtained from DuPont (Le Grand Saconnex, Switzerland). All solvents which were used for the preparation, or for the synthesis and washing of prepared monoliths; namely, sodium hydroxide (NaOH), hydrochloric acid (HCl), acetonitrile (ACN), acetone, and methanol (MeOH), were purchased from Lab Scan (Gliwice, Poland). Deionised water was supplied from a Milli-Q system (Millipore, Bedford, MA, USA). Polyimide coated (15lm thickness) fused silica capillary, 25, 50, 75, and 100 μm ID, 0.375 μm OD was purchased from Composite Metal Services Ltd., Charlestown, UK.

Instrumentation

Capillaries were filled with monomer mixture and washed using a KDS-100-CE syringe pump (KD Scientific, Inc., Holliston, MA, USA). Formation of the monolithic layer was carried out in a water bath, using a Yellow Line MST Basic hotplate with TC1 temperature controller and glassware (VWR Ltd., Dublin, Ireland). A Rheodyne 6-port switching valve (Rheodyne, Cotati, CA, USA) was used to switch between the polymerisation mixture and MeOH flows. Laminar flow studies were carried out using a PHD2000 syringe pump, purchased from Harvard Apparatus (Holliston, MA, USA). An Upchurch Model # 565 flow cell (IDEX Health and Science LLC, Washington, USA) was used to measure flow rates. Additional experiments using flow gradients used a Dionex Ultimate 3000 nanoHPLC system (Dionex, Sunnyvale, CA, USA), which was also utilised for the chromatographic separation of proteins. A SputterCoater S150B (BOC Edwards, Sussex, UK) was used for coating capillary monolithic stationary phase samples with a 60 nm gold layer. Scanning electron microscopy (SEM) analysis was performed on an S-3400N instrument (Hitachi, Maidenhead,

UK). Optical microscopy evaluation of samples was performed on a Meiji Techno EMZ-8TR stereomicroscope (Meiji Techno UK Ltd., Somerset, United Kingdom). Fluid viscosities were measured using anAntonPaarMCR 301Rheometer (Anton Paar GmbH, Graz, Austria).

Procedures

Fused silica capillaries were initially pretreated through activation of the surface silanol groups of the inner walls by sequential flushing with 1 M NaOH, deionised water, 0.1 M HCl, deionised water, and acetone. The pretreated capillary was silanised using a 50 wt% solution of trimethoxysilylpropyl methacrylate in toluene at 60 ^{0}C for 24 h. The BuMA-EDMA monomer mixture consisted of 24 wt% BuMA, 16 wt% EDMA, 59.6 wt% 1-decanol, and 0.4 wt% AIBN. The PS-DVB monomer mixture consisted of 12 wt% styrene, 12 wt% 4-vinylbenzyl chloride, 16 wt% divinylbenzene, 18 wt% toluene, 41.6 wt% 1-decanol, and 0.4 wt% AIBN (with respect to monomers). No polymerisation inhibitors were removed and monomers were used as supplied. The polymerisation mixtures were prepared as per the procedure described by Nesterenko et al. [14].

Laminar Flow Polymerisation

For the in-column polymerisation, the desired length (from 30 to 1.5 m) of silanised capillary was coiled and one end connected to a port on the switching valve which was mounted above a heated water bath.

The two inlet ports of the switching valve were connected to a syringe filled with polymerisation mixture and another syringe filled with MeOH, respectively. Both syringes were placed in a syringe pump. The coiled capillary was immersed in the water bath and the other end was left open so that the polymerisation mixture could flow through it. A typical experimental set-up is shown in Supplementary Information Figure S2.

The polymerisation mixture was pumped through the capillary at the desired flow velocities; namely, 0.25, 0.5 , or 1.0 mm s^{-1}. After flow was established the water bath was brought up to a polymerisation temperature of 60 ^{0}C.

The formation of the porous polymer layer was allowed to continue for the desired length of time, after which the water bath was evacuated and the hot water was replaced with cold water to quench any further reaction. The switching valve was also switched over to flush the capillary with MeOH to remove all unreacted monomer. Once the capillary had been thoroughly washed it was removed and dried with nitrogen.

In the above polymerisation set-up, thermal gradients, both axial and longitudinal, were avoided, as the dimensions of the capillary permitted almost instant thermal equilibration of the monomer mixture [15]. In addition, the linear flow velocity was very low, further facilitating faster thermal equilibration. Given the high thermal conductivity of fused silica, capillary dimensions and flow rate, a longitudinal thermal gradient could exist only at the very column entrance, which for each column produced was later removed and discarded. Therefore, in the current work, thermal gradients could have no measurable effect upon monolithic layer thickness.

Theory

The method proposed herein uses the effect of laminar flow to build up a porous polymer layer on the inside of a fused silica capillary through thermal polymerisation. Owing to the small diameter of the fused silica capillaries (typ. 10–400 μm ID), fluids flowing through them exhibit extremely low Reynolds numbers. Furthermore, these types of capillary possess remarkably smooth inner surfaces, with root mean square roughness values of approximately 0.4–0.5 nm [16]. The combination of such a narrow, smooth bore and low Reynolds number, results in highly laminar flow within the capillary, causing the effective formation of 'stream tubes' within the flowing liquid (see Fig. 1).

Figure 1 shows a schematic of fluid flowing through a capillary and the various forces affecting its flow structure and profile. Within Fig. 1: y, distance above capillary wall; dy, thickness of each shearing layer; dx, distance moved by each shearing layer relative to the adjacent layer; u, linear velocity of any layer; du, increase in linear velocity between any two adjacent layers; p, force due to pressure; dp, capillary pressure drop between up and downstream end; τ, shear stress; dτ, increase in shear stress over adjacent layer.When a fluid flows over a surface, the layer in contact with the surface will have a linear flow ≈0 mm s-1, because the fluid is effectively attached to the surface.

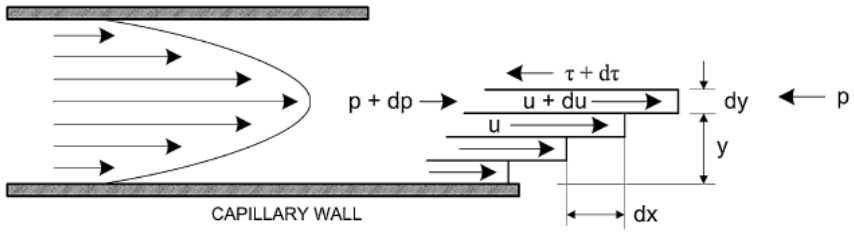

Fig. 1 Laminar flow model for a narrow bore capillary (adapted from Ref. [17])

The layers of fluid above the surface are moving and so shearing layers will exist within the fluid, each layer moving a distance, dx, in a time, dt, relative to the layer outside it.

The ratio dx/dtwill vary with the change in flow velocities between layers, giving du = dx/dt, inducing a shear stress (τ) between the layers which corresponds to a shear strain, γ. Shear strain can be defined as [17]:

$$\gamma = \frac{\text{Deformation of layer}}{\text{Thickness of layer}} = \frac{dx}{dy} \qquad (1)$$

It follows that the rate of shear strain will be:

$$\dot{\gamma} = \frac{\gamma}{dt} = \frac{dx}{dt\,dy} = \frac{du}{dy}$$

For Newtonian fluids the rate of shear strain is directly proportional to the shear stress between the layers in the fluid. The constant of proportionality in this case is the dynamic viscosity of the fluid, l, giving:

$$\tau = \dot{\gamma} \times \mu$$

Thus, the dynamic viscosity of the fluid can be defined as:

$$\mu = \frac{\text{Shear stress}}{\text{Rate of shear}} = \frac{\tau}{\dot{\gamma}} = \tau \frac{dy}{du} \qquad (4)$$

From Eq. (4), it can be seen that for a given dynamic viscosity, μ, and flow velocity, u, the shear stress, τ, will change across the radius of the capillary, reaching a maximum at the capillary wall, where the effective flow velocity, u $\approx$0 mm s^{-1} (see Supplementary Information Figure S3a).

During polymerisation, the rate of polymer growth at the wall of the capillary is heavily dependent on the liquid shear stress at the capillary walls. During no-flow conditions, polymer growth will initially start at the capillary walls. During no-flow conditions ,polymer growth will initially start at the capillary walls as the silica has a high thermal conductivity ($\sim$1.4 W m^{-1} k^{-1}) [18].

However, since there is no flow, the shear stress in the fluid will be zero and provided the entire capillary has equilibrated to polymerisation temperature, polymer growth will occur throughout the mixture. Under laminar flow conditions, this cannot occur and net growth of the polymer layer is from the capillary walls inwards towards the centre of the capillary, see Supplementary Information Figure S3b.

Applying heat energy to the outside of the capillary with a flowing polymerisation mixture will subject the outer streams of the mixture to more energy due to the lower flow velocity at the capillary walls. Since the linear flow rate decreases radially in the capillary due to laminar flow, free radicals and short polymer chains are also removed more slowly at the capillary wall zones than at its centre.

Theoretically, this polymerisation process should be scalable between capillaries of different diameters. However, although the scaling process is straightforward, it is first necessary to calculate the flow velocity which provides the optimum relative shear stress at the capillary walls for the desired layer thickness and morphology. This can be done experimentally, but once calculated the process can be scaled for any given polymerisation mixture.

Figure2 shows a comparison between shear stresses within the liquid in a 50 µm ID (solid black line) and 100 µm ID (broken black line) capillary for a given volumetric flow rate, FR = N. The shear stress in the Ø100 µm ID capillary at the desired flow rate, FR = n, is also shown. In scaling from a 50 µm ID capillary to 100 µm ID, the relative shear stresses in the polymerisation liquid must be considered.

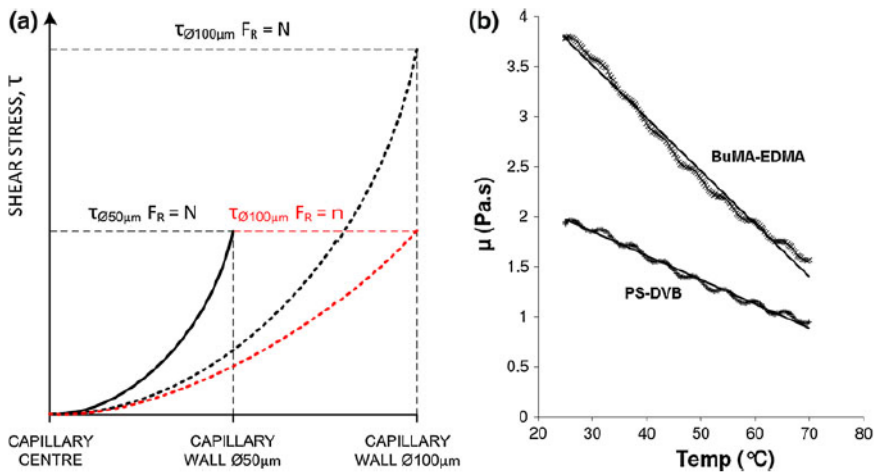

Fig. 2 **a** Comparison between shear stress, τ, across capillary bore sizes of Ø50 and Ø100 µm at a given flow rate, $F_R = N$, **b** comparison of dynamic viscosities, µ, for BuMA-EDMA and PS-DVB polymerisation mixtures between 25 and 70 °C

Assuming that the polymerisation mixture and temperature are held constant (and thus the liquid viscosity), then the volumetric flow rate for the larger diameter capillary must be adjusted to ensure that the shear stress at the capillary wall for the larger bore capillary (broken red line) equals that of the smaller diameter capillary. This should give approximately the same thickness and porosity of layer coating for the larger capillary as for the smaller capillary.

Figure 2b shows a comparison of the dynamic viscosities for the BuMA-EDMA and PS-DVB mixtures. The influence of fluid viscosity on the formation of the porous layer will be discussed later. It is important to note that changes in fluid viscosity close to the walls (and later close to the layer boundary) due to the formation of the polymer layer further enhances the laminar flow effect.

Results and Discussion

Porous Layer Morphology

Following each polymerisation experiment, 10 samples of the capillary were removed, washed, and evaluated via SEM. The average pore and globule sizes were measured (n = 50) and plotted against flow velocity, u. This was carried out for both BuMA-EDMA and PS-DVB polymerisation mixtures. Figure 3 shows the recorded pore and globule sizes in response to flow velocity for these two polymer phases. The responses show a very clear rapid decrease in the layer porosity with increasing flow velocity, and at velocities of 1.0 mm s^{-1} and above, the layers contained no observable pore structure whatsoever.

Figure 3 also shows how the average globule size similarly decreases with increased flow velocity. However, it is also interesting to note that the variability (%RSD) of the porestructuredecreasessignificantlyforbothpolymersfrom 0.25 to 0.5 mm s-1, showing that it was possible to tightly control the layer morphology under these conditions. Figure 4 shows SEM images of two 100-lm ID capillaries containing porous PS-DVB layers obtained at different flow velocities, (a) 0.5 mm s-1 and (b) 0.25 mm s-1, each polymerised for 90 min at a temperature of 60 C.

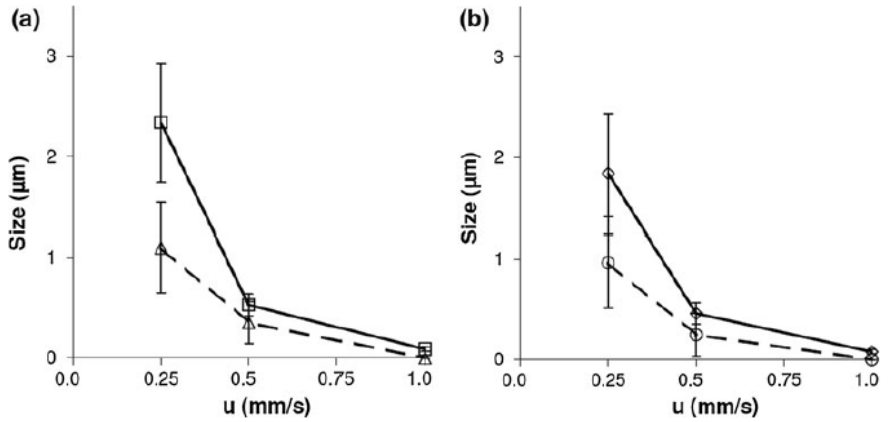

Fig. 3 Pore and globule sizes (*dashed and solid lines*, respectively) for **a** BuMA-EDMA and **b** PS-DVB porous layers in relation to various flow velocities of the polymerisation mixture

Layer thicknesseswereмeasuredtobeintheorderof1.2and3 μm, respectively. The difference in layer morphology is clear from close inspection of Fig. 4b, which shows that although 0.25 mm s^{-1} produces a much larger pore and globule structure, it also develops a much less homogenous layer. The layer thickness %RSD for (b) was measured at 51 %, as compared to just 20 % for (a).

Figure 4c, d illustrates the more homogeneous phase coverage that can be achieved at the flow velocity of 0.5 mm s-1 within the 100-lm ID capillary (temperature of 60 0C). Under these conditions, homogenous PS-DVB layers of 4 and 5 lm, with desired porous monolithic type polymer structure were obtained.

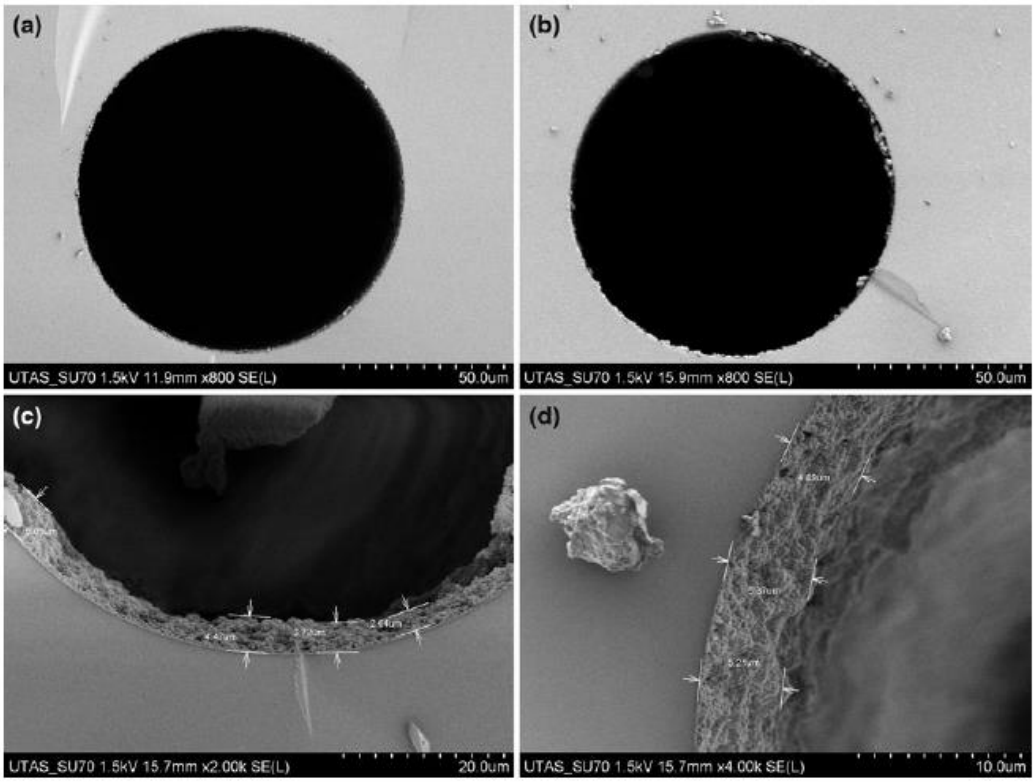

Fig. 4 SEM images of porous PS-DVB layers formed within 100 µm ID at different flow velocities, **a** 0.5 mm s^{-1} and **b** 0.25 mm s^{-1}. Polymerisation time was 90 min for each sample at a temperature of 60 °C. *Insets* **c** and **d** show SEM images of 4 and 5 µm layers formed at a flow velocity of 0.5 mm s^{-1} in 100 µm ID capillary at a temperature of 60 °C. Polymerisation time was 135 min

It is clear that once a layer of polymer begins to form on the surface of the capillary two further factors will begin to affect the polymerisation process. Firstly, the layer (regardless of its porosity) will reduce the effective ID of the capillary, causing the flow velocity to increase for a given volumetric flow. Figure S4 in Supplementary Information shows a simple comparison of the percentage change in flow velocity, u, for layer thicknesses between 1 and 10 μm within capillaries of ID between 50 and 200 μm. For simplicity, here the effects of layer porosity and increased surface friction have been ignored; however, these additional effects would be negligible.

Figure S4 shows how this increase in linear velocity with stationary phase growth will be most apparent in smaller ID capillaries, making this approach difficult to control for capillaries smaller than 50 μm ID. It can be seen that a layer of 4-μm thickness will result in a 44 % increase in the fluid linear velocity for a 50 μm ID capillary, and just a 17 % increase in a 100 μm ID capillary. The increased flow velocity will negatively affect the rate of layer growth and the morphology of the layer structure.

If the flow velocity increases too much, it may cause part of the stationary phase layer to break away, potentially blocking the column. Should this take place, complete polymerisation will rapidly occur with the process no longer in control. For these reasons, it is recommended that a flow gradient be used for capillaries with an ID less than 75 μm, the volumetric flow rate being reduced as the layer builds to ensure a constant flow velocity.

A secondary effect of layer growth is a reduction in laminar flow and an increase in turbulent flow, which will also have an increasingly negative impact on the further growth of the layer. Turbulent flow will result in a loss of layer homogeneity, and this can be seen from the high %RSD (typically[35 %) obtained for thicker layers. This phenomenon is demonstrated in Supplementary Information Figures S5a and S5b, showing examples of a 100-μm ID column with a 10–15 μm layer which exhibited layer non-uniformity, possibly due to turbulent flow during the polymerisation process. Layer homogeneity is lost since turbulent flow may remove shorter polymer chains that are weakly attached to the layer and deposit them elsewhere, resulting in a non-uniform structure.

Once this process begins, it will continually worsen as the layer becomes less and less uniform resulting in increased turbulence within the polymerisation mixture. It is unlikely that this effect is due to shear fracture during early formation of the layer as the effect would be observed in layers of all thickness and this is not the case. As the layer grows the level of shear stress acting upon the layer will increase depending on the layer thickness and the ID of the capillary. Once turbulent flow begins there will be localised areas of high and low shearing stress, and this is the probable cause of the effect as it is only observed in localised areas of thicker layers. The Reynolds number will constantly change due to the layer porosity, morphology and effective surface roughness and so the calculation of turbulent flow under these conditions is beyond the scope of this work as it would present an extremely difficult task.

Controlling Layer Thickness

A study showing the possibility of precise control of layer thickness of two types of monolithic phases, fabricated in capillaries of various ID and using different flow velocities was performed. In Fig. 5 comparisons between layer thickness and flow velocity for PS-DVB in 50, 100 and 150 lm ID capillaries, and BuMA-EDMA in 50 and 100 lm ID capillary are presented.

For all five plots, it can be seen that the rate of layer growth increases dramatically above 60 min for PS-DVB, and above approximately 90 min for BuMA-EDMA. Moreover, for Fig. 5a–c that there is good correlation between the layer thicknesses, illustrating how the process is easily scalable to larger diameter capillaries. For example, after polymerisation for 135 min, layer thicknesses for a flow velocity of 0.5 mm s-1 in capillary diameters of 50 , 100, and 150 lm ID were measured at 2.9, 3.5, and 4.5 lm, respectively.

Figures 5a–c also confirm that the optimum flow velocity was 0.5 mm s-1, giving a homogenous PS-DVB layer thicknesses of approximately 18 and 14 lm after 150 min in the 100 lm ID and 150 lm ID capillaries, respectively.

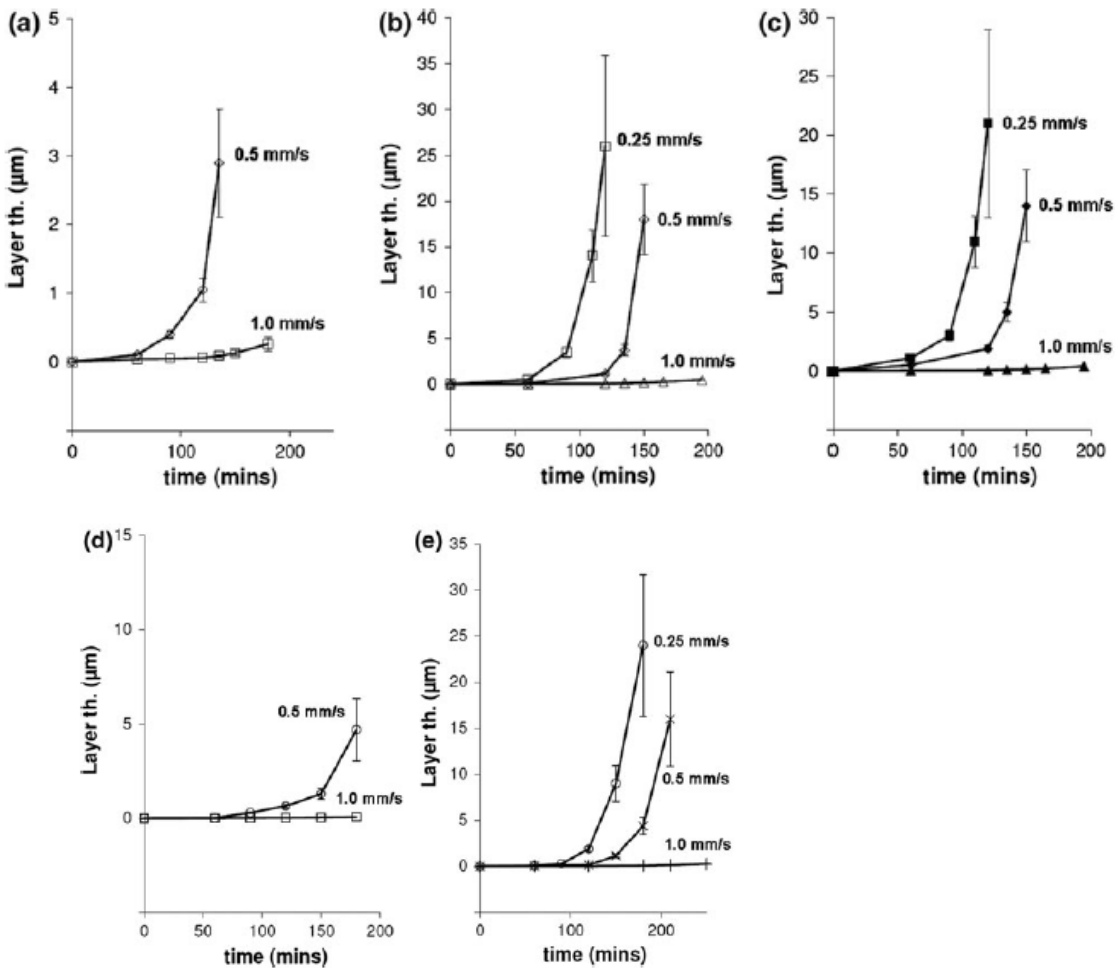

Fig. 5 Comparison of layer thickness for a PS-DVB porous layer fabricated in a **a** 50 µm ID, **b** 100 µm ID and **c** 150 µm ID capillary, and a BuMA-EDMA porous layer fabricated in a **d** 50 µm ID and **e** 100 µm ID capillary at linear flow rates of 0.25, 0.5, and 1.0 mm s^{-1}. Polymerisation temperature was 60 °C

The relationship between flow velocity and layer thickness for a BuMA-EDMA layer in 50 and 100 μm ID capillaries is shown in Fig. 5d, e. In addition, there is good correlation between the rates of layer growth in the two capillary sizes. As per the PS-DVB layer, the %RSD value for the 50 μm ID capillary is slightly higher (35 % compared with 21 % for the 100 μm ID capillary) at 0.5 mm s^{-1} flow, probably due to increased turbulence in the fluid due to layer build up.

It is interesting to note that the layer formation occurred more slowly in case of the BuMA-EDMA phase, when compared with PS-DVB. The formation of a 3 lm layer in 50 lm ID capillary at a flow velocity of 0.5 mm s^{-1} took ~180 min for BuMA-EDMA, and only 120 min for PSDVB. This may be partially related to the BuMA-EDMA polymerisation mixture exhibiting a higher viscosity (see Fig. 2b), which causes an increase in shear stress [see Eq. (3)] and slows down the layer formation compared with the PS-DVB mixture. Clearly, the viscosity of the polymerisation mixture plays a significant role, as generally methacrylate based monomers are characterised by a higher reaction rate of the chain growth step, as compared to styrene-based monomers [19].

Initial Chromatographic Performance Evaluation

One of the key advantages of monoPLOT columns is high flow-through permeability, which makes them a promising type of stationary phases for low pressure chromatography. Although PLOT columns have been applied in LC separation [5], the PS-DVB columns were based upon 10-lm ID fused silica capillary of 4.2 m length. These long PLOT columns showed great efficiency and peak capacity for the separation of protein digests, however, the separation time and backpressure were extremely high.

Herein, as expected, a significant reduction in column length and increased internal diameter (300 9 0.1 mm ID, layer thickness 2 μm), as compared to the above study [5] ,obviously resulted in much lower peak capacities and efficiencies. However, the low pressure liquid chromatographic separations obtained demonstrate considerable potential for further optimisation, including the formation of columns of greater length. Initial chromatographic performance was investigated by separating a mixture of 10 proteins using a simple 35 min acetonitrile–water gradient (0.1 % TFA) from 1 to 90 % ACN. Column temperature was kept constant at 22 C. The flow rate applied was 1 μL min^{-1}, and importantly the backpressure was only 0.6 MPa. This initial low pressure separation obtained on the above column is shown in Fig. 6.

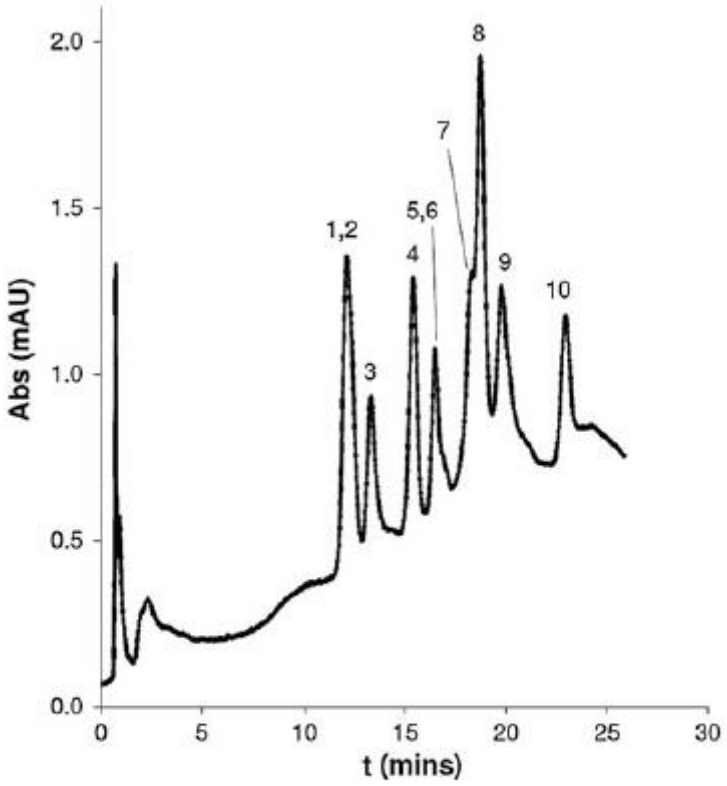

Fig. 6 Separation of ten proteins (*1* insulin, *2* ribonuclease B, *3* trypsin, *4* ribonuclease A, *5* cytochrome C, *6* myoglobin, *7* horseradish peroxidase, *8* phosphatase B, *9* carbonic anhydrase, *10* concanavalin A) under ACN–water gradient conditions: from 1 to 90 % acetonitrile (0.1 % TFA) over 35 min, flow rate 1 µL min^{-1}. Column: 300 mm × Ø50 µm ID PS-DVB monoPLOT column, layer thickness ~2 µm. UV detection at 214 nm

Conclusions

A new method for the fabrication of thermally initiated monoPLOT columns with fine control over the polymerisation process was described. The proposed method allows precise control of both layer thickness and morphology, and is potentially applicable to a variety of polymer phases within a large variety of capillary formats, significantly expanding the range of monoPLOT columns available for application within both GC and LC. Herein, the process was demonstrated with two polymers; namely, PS-DVB and BuMA-EDMA, and on three column sizes, showing the process to be scalable and with a high dependency on fluid shear stress within the capillary. The relationship between flow velocity of the fluid and both layer thickness and morphology was demonstrated at a polymerisation temperature of 60 C. An initial chromatographic evaluation was shown for the low pressure separation of a text mixture of proteins.

Acknowledgments :The authors would like to acknowledge financial support from Science Foundation Ireland for the Irish Separation Science Cluster award (Grant No. 08/SRC/B1412).

References

1. Golay MJE (1958) Gas chromatography. Academic Press, New York
2. de Zeeuw J (2011) LC-GC Eur 24:38–45
3. Collins D, Nesterenko E, Brabazon D, Paull B (2012) Anal Chem 84:3465–3472
4. Nischang I, Brueggemann O, Svec F (2010) Anal BioanalChem 397:953–960
5. Luo Q, Rejtar T, Wu SL, Karger B (2009) J Chromatogr A 1216:1223–1231
6. Huang X, Zhang J, Horvath C (1999) J Chromatogr A 858:91–101
7. Tan ZJ, Remcho VT (1998) J Microcolumn Sep 10(1):99–105
8. Tan ZJ, Remcho VT (1997) Anal Chem 69(4):581–586
9. Chuang SC, Chang CY, Liu CY (2004) J Chromatogr A 1044:229–236
10. Shen TC (1992) J ChromatogrSci 30(6):239–240
11. Banu I, Bildea S, Bozga G, Puaux JP (2009) Stud UnivBabesBolyaiChem 54(1):227–241
12. Castro JM, Lipshitz SD, Macosko CW (1982) AIChE J 28(6): 973–980
13. Wehner JF (1978) Chem React EngHoust. doi:10.1021/bk-19780065.ch012
14. Nesterenko EP, Nesterenko PN, Connolly D, Lacroix F, Paull B (2010) J Chromatogr A 1217:2138–2146
15. Collins DA, Nesterenko EP, Connolly D, Vasquez M, Macka M, BrabazonD, Paull B (2011) Anal Chem 83(11):4307–4313
16. Cifuentes A, Diez-Masa JC, Fritz J et al (1998) Anal Chem 70(16):3458–3462
17. Fox R, McDonald A, Pritchard P (2009) Introduction to fluid mechanics, 7th edn. Wiley, New York
18. http://www.polymicro.com. Accessed November 2012
19. Odian G (1981) Principles of polymerization, 2nd edn. Wiley, New York