



Micro-bore titanium housed polymer monoliths for reversed-phase liquid chromatography of small molecules

Ekaterina P. Nesterenko^a, Pavel N. Nesterenko^b, Damian Connolly^a, Flavie Lacroix^c, Brett Paull^{a,*}

^a Irish Separation Science Cluster, National Centre for Sensor Research, Dublin City University, Glasnevin, Dublin 9, Ireland

^b Australian Centre for Research on Separation Science, University of Tasmania, Hobart, Tasmania 7001, Australia

^c Centre d'études supérieures d'ingénieurs, 297, Rue de Vaugirard-75015, Paris, France

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ABSTRACT

A new method for the fixation of polymethacrylate monoliths within titanium tubing of up to 0.8 mm I.D. for use as a chromatographic column under elevated temperatures and pressures is described. The preparation of butyl methacrylate–ethylene dimethacrylate-based monolithic stationary phases with desired porous structures was achieved within titanium tubing with pre-oxidised internal walls. The oxidised titanium surface was subsequently silanised with 3-trimethoxysilylpropyl methacrylate resulting in tight bonding of butyl methacrylate porous monolith to the internal walls, providing stationary phase stability at column temperatures up to 110 °C and at operating column pressure drops of >28 MPa. The titanium housed monoliths exhibited a uniform and dense porous structure, which provided peak efficiencies of up to 59,000 theoretical plates per meter when evaluated for the separation of small molecules in reversed-phase mode, under optimal conditions (achieved at 15 μL/min and temperature of 110 °C for naphthalene with a retention factor, $k = 0.58$). The developed column was applied to the reversed-phase isocratic separation of a text mixture of pesticides.

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1. Introduction

Based upon their reported high permeability and acceptable separation efficiencies, the use of monolithic porous media has now been demonstrated in most modes of chromatographic analysis including GC [1], HPLC [2], IC [3,4], CEC [5] and SPE [6]. Depending upon the scale and specific goals of the chromatographic application monolithic porous bodies of various geometry and shape can be prepared and housed, with the most common applications involving monolithic rods [7] and disks [8], and monoliths housed within capillaries [9], micro-channels [10] and pipette tips [11]. However, there remains some significant size-related limitations in the use of monolithic phases for chromatographic applications, which are still the subject of a considerable body of research. For example, organic polymers (polystyrene-divinylbenzene (PS-DVB), polymethacrylate (PMA), etc.) and various inorganic oxides represent the two main material classes which have been used in the preparation of monolithic porous columns for liquid chromatography. However, the complex combination of different physico-mechanical properties and limitations of the above substrates, such as varying degrees

of rigidity and fragility (cracking, shrinking and swelling), pH and temperature stability, surface area and pore geometry, and wall adhesion chemistry, places some practical limits on the size and geometry of columns which can be produced, especially at the level of commercial production.

Rigid silica-based rods of diameter greater than 2 mm and length 10–15 cm, can be prepared by established sol-gel technology followed by calcination at high temperatures. Subsequent thermo-shrinking based cladding of these rods is used for the preparation of commercial HPLC columns [12]. On the capillary end of the scale, the significant shrinking of hydrated silica gel during calcination, and the fragility of the final silica monolith produced, limits the possibilities of *in situ* preparation of columns in standard fused silica (FS) capillaries of I.D. less than 100 μm. However, the activated/hydroxylated internal walls of FS capillaries are involved in the sol-gel process of the monolith body formation, providing strong bonding of the monolith body within such FS capillaries of I.D. of >100 μm.

In situ polymerisation in various sizes of FS capillaries is a common approach for the preparation of organo-polymer monolithic columns [2,13]. However, difficulties again arise in the preparation of long columns of internal diameter greater than 1–2 mm. The stable bonding of the polymer rod within the column housing is a key challenge, particularly for higher pressure and temperature modes of chromatography. Current commercially available organo-polymer-based columns can generally only operate at

* Corresponding author at: National Centre for Sensor Research, Dublin City University, School of Chemical Sciences, Glasnevin, Dublin 9, Ireland.

Tel.: +353 1 7005060; fax: +353 1 7005503.

E-mail address: Brett.Paull@dcu.ie (B. Paull).

backpressures up to 20 MPa and temperatures up to 70 °C [14]. This of course depends upon the rigidity of the organo-polymer matrix, the internal wall surface area and, not less importantly, on the strength of bonds between the polymer matrix and the internal walls of the column housing. Thus, Courtois et al. [15] studied various techniques for surface pretreatment and attachment of anchoring groups to fused silica capillaries surface and showed that silanisation technique is of extreme importance as the strength of monolith attachment depends on the chemistry utilised to obtain fixation. Other than that, the strength of attachment also depends on the chemical nature of the column housing itself.

An advantage of FS capillaries as a column housing is the high density of silanol groups on the capillary wall which can be fully activated after alkali or acidic treatment [16]. The fully activated silica surface contains 4.6 silanol groups/nm². Obviously, in the case of the *in situ* preparation of a silica monolith within a FS capillary, all available silanols are involved in the sol-gel synthesis, which provides very strong bonding of the silica structure to the wall. Similar bonding of organo-polymer monoliths within FS capillaries needs activation of the internal walls with silanes containing reactive groups. Due to steric hindrance only 50–60% of surface silanols can be converted into reactive functional groups via such treatment although this amount is also sufficient to hold organo-polymer monoliths within the FS capillary under standard chromatographic conditions. The type of the reactive functional group used for the bonding of organo-polymer monoliths depends on the type of polymer monolith being prepared. Epoxy- or oxirane groups are often used for the preparation and bonding of glycidylmethacrylate (GMA) based polymers in FS capillaries [17], and vinyl- groups utilised for PS-DVB polymers.

Clearly, the covalent fixation of the organo-polymer monolith to the silica walls is an extremely useful advantage of using a FS capillary column format in monolithic chromatographic applications. However, the use of glass column housings of larger internal diameters has not been significantly developed due to problems of column handling and strength issues related to operation at higher pressures. Additionally, poly-ether-ether ketone (PEEK) and common stainless steel (SS) tubes have also been largely avoided, as in both cases the arrangement of chemical bridges between the bulk monolith and walls is not trivial. In the case of SS the modification of the surface with silanes is not viable due to the poor hydrolytic stability of the Me–O–Si–C bonding, where Me includes various metals such as nickel, iron, chromium from the stainless steel composition. The introduction of reactive functional groups at the surface of PEEK is also highly problematic and is not yet well demonstrated in the literature. The applicability of the aforementioned cladding technique used for silica-based rods is restricted by the relative softness of the organo-polymer monoliths and can only be used in the production of disks or relatively short columns.

In this paper a new alternative column housing for organo-polymer monoliths is presented, which can help overcome some of the above limitations. For the first time surface oxidised titanium tubing of 0.8 mm I.D. has been evaluated as a suitable column housing material for organo-polymer monoliths, demonstrating highly stable surface to monolith bonding strength, structural and thermal stability of the *in-column* polymerised monoliths, and very encouraging chromatographic performance for small molecules. Within the titanium tubing a thin layer of titanium oxide can be formed by the simple oxidation of the titanium wall surface, which can be further modified using silane chemistry similarly to a FS surface, providing a stable Ti–O–Si-type of bonding, which can be both stable and readily amenable to further bonding with the organo-polymer matrix. The *in-column* polymerisation parameters for formation of monoliths of stable pore structure for efficient separation of small molecules are also presented.

2. Experimental

2.1. Chemicals and reagents

Butyl methacrylate, ethylene dimethacrylate, 1-decanol and 3-trimethoxysilylpropyl methacrylate were all purchased from Sigma–Aldrich (Gillingham, UK), as were sodium hydroxide and hydrochloric acid. The thermal initiator 1,1'-azobisisobutyronitrile (AIBN) was obtained from DuPont (Le Grand Sacconex, Switzerland). Solvents used for the synthesis, washing of the prepared monolith and for the preparation of eluents, namely acetonitrile (ACN), methanol, acetone and toluene were purchased from Lab Scan (Gliwice, Poland). Titanium tubes (100 mm × 0.8 mm I.D.) were obtained from Carl Stuart Ltd. (Dublin, Ireland). Deionised water purified by a Milli-Q system (Millipore, Bedford, USA) was utilised throughout the experiments. The standard solutions, comprising of uracil, acetophenone, benzene, toluene and naphthalene were obtained from Phenomenex (Torrance, CA, USA).

2.2. Instrumentation

A EuroTherm 91e furnace (Carbolite Furnaces, Hope, UK) was used for the thermal oxidation of the titanium tube surface in order to provide a sufficient surface layer of titanium oxide. A SputterCoater S150B (BOC Edwards, Sussex, UK) was utilised for coating the subsequent titanium housed polymer monolith sample prior to scanning electron microscopy analysis, which was performed on a S-3400N instrument (Hitachi, Maidenhead, UK). For the chromatographic study, an Ultimate 3000 Rapid Separation HPLC system was used, comprising of a HPG-3x00RS binary pump, WPS-3000RS autosampler, TCC-3000 RS thermostated column compartment (which allows the use of temperatures of up to 110 °C) and a MWD-3000RS multiple wavelength detector (Dionex, Sunnyvale, CA, USA).

2.3. Preparation of titanium housed monolithic columns

Titanium tubes (100 mm × 0.8 mm I.D.) were placed in the furnace and then heated from room temperature to 500 °C, and kept at this temperature for 6 h. The furnace was then switched off and allowed to cool down slowly to room temperature. This was followed by activation of hydroxide groups on the surface of the oxidised titanium tube to provide reactive groups for the subsequent silanisation. This was achieved through washing with 0.2 M NaOH for 1 h. The tubes were then washed with deionised water for 30 min and a further washing step with 0.2 M HCl for 2 h. Following this the tubes were again washed with deionised water for 15 min followed by acetone and then left to dry for 1 h at 90 °C.

After the above oxidation and activation steps, the walls of the column were vinylised in order to provide covalent attachment points for the polymer monolith to the titanium surface. This was performed by reacting the column walls with 3-trimethoxysilylpropyl methacrylate, which acts as a linker between the inorganic titanium surface and organic polymer material. The pre-treated titanium tubes were silanised with 50 wt% 3-trimethoxysilylpropyl methacrylate in toluene at 90 °C for 24 h. No polymerisation inhibitors were added.

Finally, the butyl methacrylate-based monolithic stationary phase was produced using an adapted procedure of Geiser, previously shown to be both robust and reproducible [18]. Methacrylate-based monolithic stationary phases were chosen for the study due to their rigid properties and ease of preparation through direct co-polymerisation of the functional monomer and cross-linker. The monomer mixture consisted of 24 wt% of

butyl methacrylate, 16 wt% ethylene dimethacrylate, 60 wt% 1-decanol and 1 wt% AIBN (in respect to monomers). The initiator was weighed into the mixture vessel first, then the porogen (1-decanol) was added and the mixture was sonicated for 60 min to dissolve AIBN (AIBN was first dissolved in the porogen, due to the observed limited solubility of AIBN in the polymerisation mixture, thus avoiding any possible negative effects from the presence of solid phase in the polymerisation mixture, and also avoiding any possible starting polymerisation due to heating during sonication). Then the rest of the reagents were added and the mixture was vortexed for 5 min. After that the mixture was centrifuged for 13 min at 10,000 r/min. and 80% of the supernatant was taken and used to fill an oxidised and silanised titanium tube, which was placed in NMR test tube (no sediment was observed, however, centrifugation was carried out in order to remove any possible solid phase micro-contaminants, in order to avoid their affect on the polymerisation process). Prior filling the mould, polymerisation mixture was deaerated for 10 min under the flow of nitrogen. The prepared tube was placed in the water-bath at room temperature (20 °C), the temperature was slowly increased using a temperature gradient from 20 to 50 °C over a period of up to 1 h, in small increments of ~5 °C every 10 min and from 50 to 55 °C over a period of 50 min in small increments of ~1 °C. Once the temperature reached 55 °C, it was kept constant for 8 h. After the end of synthesis, the prepared column was left to cool down at room temperature overnight. To remove the column from the glass NMR test tube, it was carefully crushed followed by removal of the glass. The bulk monolith outside the column was then cut off and the column cleaned with fine sand paper. After that appropriate fittings were attached to the column and it was washed with ACN at a flow rate of 1 µL/min for approximately 100 min and then at 10 µL/min for at least 60 min. During the removal of residual monomer mixture and porogen from the pores of the monolith, the backpressure initially increased to 20 MPa and then dropped down to 3.9 MPa.

3. Results and discussion

3.1. Oxidation of titanium column housings

As titanium is highly reactive and has a very high affinity for oxygen, titanium oxide films on the surface form spontaneously and instantly when metal is exposed to air. The nature, composition and thickness of the oxide layer on the surface depends on environmental conditions. In most environments the oxide is typically TiO₂, but can also consist of a mixture of oxides, including TiO₂, Ti₂O₃ and TiO [19]. These naturally formed films on the surface of titanium are typically less than 10 nm thick, so in order to provide a sufficient layer of titanium oxide on the inner surface of the titanium tube, it was oxidised prior to silanisation. Titanium surfaces can be oxidised through chemical oxidation or thermal oxidation. Though chemical oxidation provides a consistent and reproducible titanium oxide surface layer, thermal oxidation was chosen for this procedure as it does not require the use of such chemicals as sulphuric acid and hydrogen peroxide [20]. Additionally, studies performed by Padma et al. [21] showed that at temperatures below 900 °C and oxidation duration over 3 h, a layer of titanium oxide was formed, which consisted of 98–100% TiO₂, depending on depth of the layer (0–1 µm, with the percentage of TiO₂ decreasing with layer depth), and that the composition of the oxide in this surface layer was both reproducible and consistent.

The temperature of the titanium oxidation procedure was very significant. At oxidation temperatures below 600 °C a chemically active modification of titania (anatase) exists, while at temperatures over 620–650 °C, a chemically inert modification—rutile is

formed. As the oxidation step is followed by activation of the OH-groups (typically the surface concentration of Ti–OH groups would be 4.3–4.8 groups/nm² [19]) and attachment of vinyl functional groups, it is important to have an active form of TiO₂ on the surface. So the thermal oxidation was performed at 500 °C for 6 h.

3.2. Optimisation of polymerisation temperature and duration

Another crucial step in the formation of organo-polymer monoliths with uniform porosity in titanium housing, is optimisation of both the temperature and duration of polymerisation. Initially the polymerisation step was performed at 60 °C for 24 h based upon previous studies using FS capillaries [22]. However, when the column was connected to the HPLC system, it was not possible to pump through it, with backpressures >20 MPa at 1 µL/min. No reduction in backpressure was observed after 72 h of constant washing with ACN. SEM imaging confirmed that the porosity of the stationary phase was very low. A reason for the formation of a monolith of such high density could be the high thermal conductivity of titanium, which is 21.9 W/m/K [23] (for FS this is 1.3 W/m/K [24], and for PEEK this value is only 0.25 W/m/K [25]). This high thermal conductivity results in faster heat dissipation in the titanium column. As a consequence the monomer mixture would heat up faster, providing a more rapid polymerisation and a higher temperature inside the column [26].

It is well known that in order to obtain fine control of monolith porosity, reaction parameters such as nature of porogen, the ratio of monomer to cross-linker in the mixture, polymerisation temperature and time can be varied. Here, the aim of the work was successful attachment of the polymer monolith to the titanium housing, together with demonstration of the resultant monolithic column chromatographically under elevated column temperature and pressure conditions. Therefore, in this study only the polymerisation temperature and duration were studied in detail.

The reaction rate for free-radical polymerisation is not a simple function of temperature, as the process consists of several steps [2,27,28]. The most important is the initiation rate, which is highly dependant on temperature, since the half-life of initiators decreases with increases in temperature. As a result, the decomposition rate of the initiator, the number of radicals produced, the formation of more globules, and consequently the overall polymerisation rate are higher at elevated temperatures. As the formation of new polymerisation centres is faster than the growth of globules, the supply of monomers runs low fast and the number of globules is large, but their size stays small, leading to smaller voids between globules. So essentially, higher polymerisation temperatures result in smaller pore sizes.

The duration of polymerisation also has an affect upon the pore size of the resultant monolith. With longer polymerisation times, monolith globules increase in size resulting in smaller voids between them, and as a result leading to smaller pores and higher density of the monolith [2].

For initial investigations into the temperature and polymerisation duration effects on the porosity of the monoliths, butyl methacrylate–ethylene dimethacrylate monoliths were prepared in bulk (i.e. not in titanium column housing), pre-treated as described in the experimental section. The polymerisation time was varied from 7 to 24 h at a constant temperature of 60 °C. In addition, the polymerisation temperature was varied from 54 to 60 °C for a fixed time of 7 h. SEM images of the prepared monoliths were obtained and sizes of pores and polymer globules were estimated. The semi-quantitative results are presented in Table 1. As it can be seen, the results obtained correspond with data presented in previous studies [2,7,29], i.e. higher polymerisation temperatures and longer reaction times lead to smaller pore sizes.

Table 1

The influence of polymerisation temperature and duration on approximate flow-through pore size.

Polymerisation temperature, °C at fixed time (7 h)	$d_{\text{pore}}, \mu\text{m}^a$	Polymerisation time, h at fixed temperature (60 °C)	$d_{\text{pore}}, \mu\text{m}^a$
54	1.18 ± 0.59	7	0.78 ± 0.24
55	0.78 ± 0.31	9	0.43 ± 0.23
56	0.63 ± 0.37	12	0.34 ± 0.20
57	0.71 ± 0.57	24	0.27 ± 0.16
58	0.54 ± 0.25		
60	0.43 ± 0.3		

^a Calculated for $n = 30$ pores.

3.3. Effect of gradient temperature programming during polymerisation

The preparation of monoliths with a homogeneous porous structure in capillaries is readily achieved, whereas scaling up the diameter of the monolith while still maintaining good wall-bonding is somewhat more challenging. The initial titanium housed columns prepared at the early stages of this work were found to have a non-uniform porosity distribution when cross-sections were examined by SEM, as shown in clearly in Fig. 1. The radial homogeneity of porosity is clearly quite poor, with larger pores in the central core of the monolith (section A) and smaller pores in the outer shell formed near the column walls (section B). These results correspond with the data obtained in [26] for the preparation of larger size polymer monoliths. It is likely here that the non-uniform porosity resulted from the uncontrolled increase in temperature during the preparation of large-diameter monoliths, as a significant exotherm can be observed during the polymerisation process [26] (depending on the type of the free-radical thermal initiator and the dimensions of the column, the exotherm can be up to 60 °C, which obviously has an effect on the polymerisation kinetics and the resultant structure of the monolith). However, an alternative cause of such non-homogeneity could also involve some degree of internal surface catalysed decomposition of AIBN, thus accelerating the initiation rate close to the column surface.

To investigate this further here, it was decided to run the polymerisation at a rate slow enough to allow dissipation of the heat of polymerisation, in order to attempt to provide the formation of the monolith with uniform porosity. Therefore, a slow temperature gradient was used during preparation of the monolith, which provided uniform heating of the column housing and the monomer

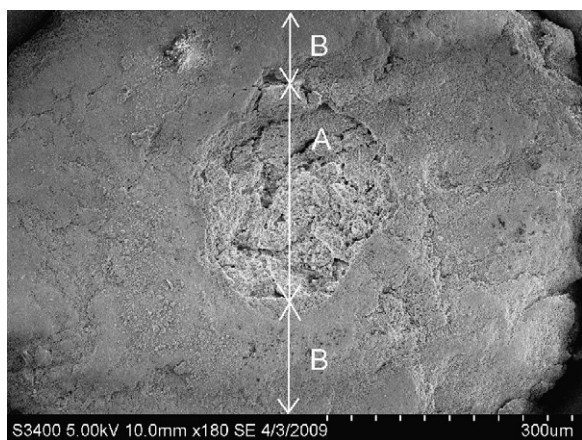


Fig. 1. Initial SEM image of radially inhomogeneous structure of a monolith thermally polymerised in 0.8 mm I.D. titanium tubing housing. Zone A corresponds to a more porous central portion of the monolith and Zone B corresponds to a non-porous exterior of the monolith.

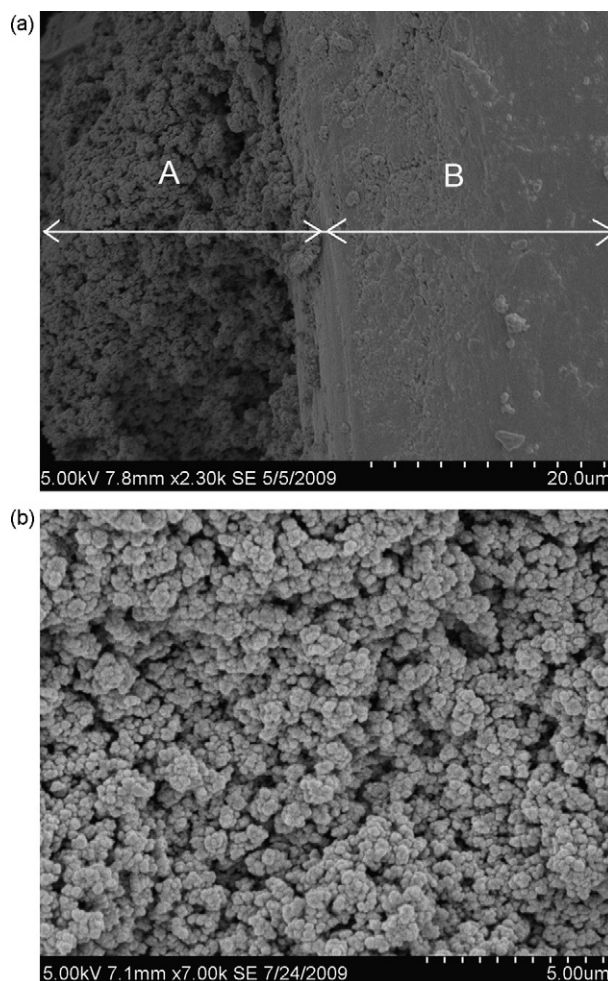


Fig. 2. SEM images of titanium housed monoliths formed using thermal gradient polymerisation. (a) SEM image showing polymer monolith bound to the inner wall of titanium column and Zone A corresponds to the polymer monolith, Zone B is the titanium column housing. (b) SEM image showing dense highly homogeneous monolithic structure.

mixture within (the application of a temperature gradient should not change the structure of the polymer inside the titanium tube if such a structure was related to surface catalysis effects rather than temperature, unless the catalysis kinetics were also affected). The temperature gradient applied was selected in such way in order to provide gradual heating. As polymerisation would not start at temperatures under 50 °C, up to that temperature the heating rate was relatively fast, and to provide better equilibration at temperatures above 50 °C, the heating rate was then significantly reduced. The image of the monolith, prepared under these conditions is shown in Fig. 2(b). The absence of a significant exotherm during slow polymerisation [26] correlates with the formation of the completely homogeneous porous structure obtained.

3.4. Scanning electron microscopy images of the monolith housed in titanium column

Using the optimised polymerisation protocols described above, a 10 mm × 0.8 mm I.D. monolith in titanium housing was prepared and imaged using SEM. Once the column was prepared, it was sonicated in acetone for 60 min and then dried for 3 h at 60 °C. The dry sample was sputtered with gold, forming a layer of approximately 60 nm. The images obtained are shown in Fig. 2. Excellent comprehensive attachment of the polymer monolith to the titanium

column wall is clearly seen in Fig. 2(a) and (b) permits an approximate evaluation of the porosity of the bulk dense monolith in the middle of the column.

The bond strength of the polymer monolith to the titanium column housing is of crucial importance in this work and an important factor in its suitability for applications under conditions generating high column pressure drops. Therefore, it is worth briefly considering here the parameters involved.

Obviously, the force of interaction, F_{int} , between the monolith body and the internal walls is proportional to the interface area:

$$F_{\text{int}} = aS = 2a\pi rl,$$

where l and r are column length and radius and a is the adhesion coefficient. In the case of an ideal incompressible porous monolith, the force pushing the monolith out from the column, F_p , can be expressed through the pressure applied to the top of the column as follows:

$$F_p = \Delta p \pi r^2 (1 - \varepsilon),$$

where Δp is the column pressure drop and ε is the porosity of the monolith expressed as the ratio of the volume of pores in 1 g of the monolith to its total volume. The monolith body would only be retained in the column housing (assuming no retaining supporting frit) when $F_{\text{int}} > F_p$ or at:

$$\frac{F_{\text{int}}}{F_p} = \frac{2a\pi rl}{\Delta p \pi r^2 (1 - \varepsilon)} = \frac{2al}{\Delta p r (1 - \varepsilon)} \geq 1.$$

The above relationship states that under similar mobile phase conditions, higher column stability or greater compatibility with higher operating pressures, can be obtained with longer columns of smaller internal diameter. The other important parameters are porosity of the column and the adhesion coefficient, a . Clearly, the highest value of the coefficient a should result from comprehensive and homogeneous chemical bonding between the monolithic rod and internal walls of the column housing.

The SEM images show that the density of the monolith was very high, providing a relatively high surface area, though the permeability of the column was expected to be relatively low. The flow-through pore diameter was estimated from the SEM data to be approximately $0.54 \pm 0.17 \mu\text{m}$ ($n=30$), and the diameter of polymer globules was found to be approximately $0.23 \pm 0.08 \mu\text{m}$ ($n=30$). These results were compared with the data reported by Causon et al. [35] and Vlakh and Tennikova [13]. The approximate globules size of the polymer monoliths, calculated by authors of the present work from published SEM images and was found to be $\sim 2.2 \pm 0.6 \mu\text{m}$ and $\sim 2.0 \pm 0.6 \mu\text{m}$, respectively, which shows that the organo-polymer monolith, obtained in the present work has a much more dense structure. The total pore volume for the titanium housed column was estimated from the determined dead time value and was calculated to be 61% of the total volume of the column.

3.5. Chromatographic evaluation of the titanium house monolith

Rapid and high throughput separations can be achieved either through using monolithic stationary phases with high permeability, allowing the use of elevated flow rates [30], or through using sub- $2 \mu\text{m}$ particles [31], but the main disadvantage in the latter case is a very high backpressure. Another option to provide faster separations is the use of higher temperatures, as the viscosity of the eluent would reduce resulting in an overall decrease in backpressure, providing not only the possibility of the use of higher flow rates, but also higher diffusion of the analyte in the eluent and stationary phase, enabling improved mass transfer. However, the use of aqueous based mobile phases at elevated

temperatures can affect the stability of silica-based monoliths, or the stability of the bond between polymer monoliths and the walls of FS capillaries. A number of studies have been published which examine the stability of different stationary phases at higher temperatures [32,33]. Andersen et al. [33] showed that a poly(styrene-divinylbenzene) monolithic stationary phase was stable over time (400 h) at 80°C and no change in retention of analytes was observed, while at 120°C , a 20% decrease in retention factor was observed after 100 h. The authors also reported that a small void ($<0.5 \text{ mm}$) was observed at the column inlet when the columns tested at 120°C were opened after use. Eeltink et al. [34] also demonstrated similar results, where he demonstrated the application of poly(styrene-divinylbenzene) monolith at 80°C . Causon et al. [35] studied the stability of a poly(divinylbenzene) monolithic column when using pure water at temperatures up to 250°C and showed that the monolith was stable for up to 30 h of operating at 220°C . As for commercially available monolithic columns, their thermal stability range varies from 40 to 80°C , for example, for the poly(styrene-divinylbenzene) based ProSwift columns maximum operating temperatures of 80°C are recommended [36], while CIM disks, depending on the type of monolith housed, can be operated at temperatures only up to $40\text{--}50^\circ\text{C}$ [37]. In the current work the titanium housed monolithic columns were constantly run at column temperatures of between 100 and 110°C . During this chromatographic evaluation work, no significant change in retention times for test analytes was observed, this amounting to $\sim 500 \text{ h}$ of constant use.

The reversed-phase monolithic columns, prepared as described above were characterised using with a number of approaches, such as column stability tests at high temperatures and backpressures, sample loading capacity, and backpressure dependence on the eluent flow rate.

3.5.1. Temperature, flow rate and column generated backpressures

Using ACN as the mobile phase, with a flow rate of $10 \mu\text{L}/\text{min}$, column temperatures were slowly increased to 110°C with incremental steps of 5°C . At each step the column was left to equilibrate until constant backpressure was reached. A decrease in column generated backpressure was observed with an increase of the column temperature, due to the decrease in mobile phase viscosity [38]. As the monoliths obtained in this work were very dense, the backpressures were relatively high even at low flow rates. Thus increasing column temperature was used to allow increased flow rates, to obtain optimum separation efficiency and decrease separation time. With an increase of the column temperature from 25 to 110°C , column generated backpressure dropped by 3.5 MPa.

To study the influence of the flow rate on the column backpressure, ACN and water were used as mobile phases, with a column temperature of 100°C . The flow rate was gradually increased with an increment of $1 \mu\text{L}/\text{min}$ and the backpressure was allowed to stabilise at each flow rate value. Fig. 3 shows that backpressure linearly increases with the increase in flow rate, clearly demonstrating monolith and bonding stability over this range. It was shown that the backpressure increases from approximately 1.0 and 1.2 MPa for water and ACN at $1 \mu\text{L}/\text{min}$, respectively, to 18.1 and 24.9 MPa for the same mobile phases respectively at the flow rate of $22 \mu\text{L}/\text{min}$. The higher pressure seen with ACN compared to water is possibly related to a different degree of swelling of the prepared polymer monolith in the more non-polar solvent, which appears more significant than simply the relative viscosities of the two mobile phases. The same linear dependence of backpressure on the mobile phase flow rate was observed for a butyl methacrylate–ethylene dimethacrylate monolithic column ($210 \text{ mm} \times 0.32 \text{ mm I.D.}$) with a similar composition (60% porogen, 40% monomer mixture), and it was also shown that the backpres-

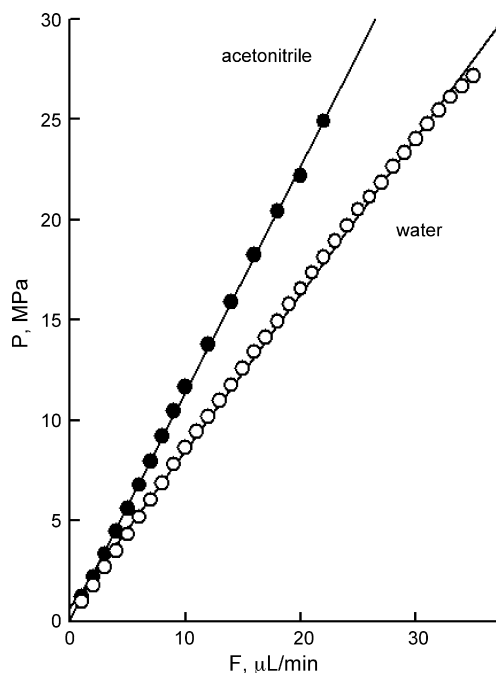


Fig. 3. Column generated backpressure dependence on flow rate at 100 °C with mobile phases of ACN ($R^2 = 0.999$) and water ($R^2 = 0.999$).

sure increased from approximately 1.2 MPa at 1 $\mu\text{L}/\text{min}$ to 3.0 MPa at 4 $\mu\text{L}/\text{min}$ using acetonitrile–water mixture of 65–35% [17].

3.5.2. Separation reproducibility

In order to investigate column stability and chromatographic reproducibility under conditions of high temperature and pressure, a standard mixture of uracil, acetophenone, benzene, toluene and naphthalene was injected repeatedly and continuously over a 55-h period. Though the separation time for the mixture was approximately 10 min, the mixture was injected once per hour, so that a total of 55 repeat injections were carried out, equivalent to over 1200 column volumes. The resultant chromatograms from this reproducibility study, are presented in Fig. 4, showing every 5th chromatogram from up to run 55, overlaid. As it can be seen, the column was stable over this period of time. The relative standard deviation values (RSD) for such chromatographic parameters as retention time (t_r), peak asymmetry (A_s) and efficiency (N) were found to be 0.8–1.1%, 1.1–3.4% and 1.4–5.6%, respectively. The calculated RSD values were found to be smaller than those obtained by Holdsvendova et al. [39] for methacrylate–ethylene dimethacrylate-based monolithic capillary columns, which were 1.7–4.8% for the retention factor (k), 2.7–3.9% for A_s and 1.6–16% for N .

Low RSD values indicate that after prolonged use of the column at higher temperature and pressure, no changes in the column structure occurred (e.g. cracks, voids) and that the polymer monolith did not detach from the inner column wall and was still strongly bound to it. It is also important to note that during this experiment no change in the column backpressure was observed, which also suggests that no detachment of the monolith occurred.

3.5.3. Evaluation of separation efficiency

The efficiency of the titanium housed monolithic column, was evaluated under varying eluent flow rates. A van-Deemter plot was constructed for naphthalene peak (100 ppm, 0.2 μL injection volume), with eluent flow rates ranging from 1 to 18 $\mu\text{L}/\text{min}$. The plot is shown as Fig. 5. The velocity range was limited by the backpressure, although the data indicated the minimum H values were to be

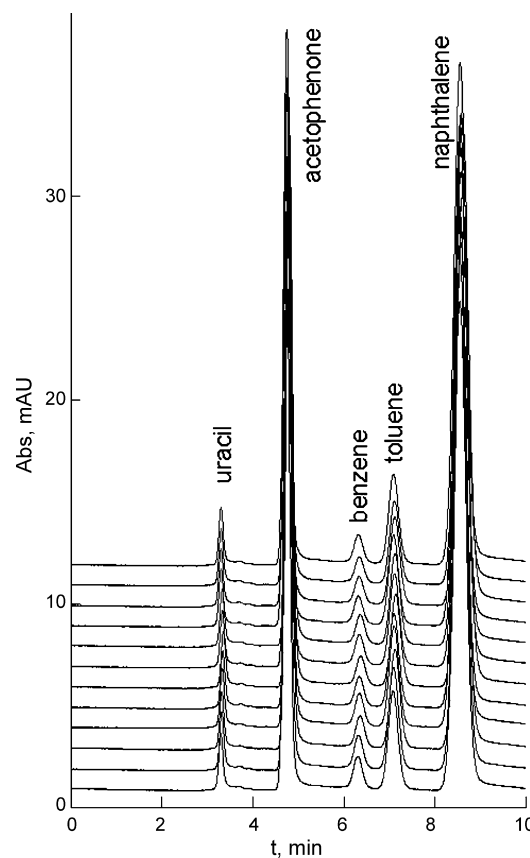


Fig. 4. Overlaid 11 chromatograms (each 5th chromatogram) of 55 consecutive injections of a standard mixture on a butyl methacrylate–ethylene dimethacrylate polymer monolith housed within a 100 mm \times 0.8 mm I.D. titanium column. Mobile phase = 60% ACN–40% water, $F = 10 \mu\text{L}/\text{min}$, column temperature = 100 °C. UV detection at 254 nm.

found at mobile phase velocities of between 0.6 and 0.8 mm/s (15 and 18 $\mu\text{L}/\text{min}$). Under these flow conditions backpressures were relatively high (e.g. 34 MPa at 18 $\mu\text{L}/\text{min}$). At the optimal flow rate of 15 $\mu\text{L}/\text{min}$, the efficiency calculated for the naphthalene peak was $\sim 59,300 \text{ N}/\text{m}$.

The observed efficiency data over the range of mobile phase velocities studies, compared favourably to that reported by Siouffi [40], for various polymer-based monoliths, and to that reported by Moravcova et al. [22], who studied van-Deemter's curves for butyl methacrylate–ethylene dimethacrylate-based monolithic capillary columns prepared using different porogens.

The influence of the injection volume on peak efficiency was also studied. Chromatographic conditions were as follows: the eluent used was 90% acetonitrile–10% water, flow rate was 10 $\mu\text{L}/\text{min}$ and the column temperature was 60 °C. For this study the 100 ppm naphthalene solution was injected onto the column with injection volumes ranging from 0.03 μL to 2 μL . Reduction of injection volume can reduce peak broadening since column overloading is eliminated, and as anticipated, Fig. 6 shows the increase in peak efficiency with a decrease in injection volume. Some small variation in the trend was seen at the lowest injection volumes, possibly due to uncertainty introduced from operation of the autosampler at the very lowest of its range, although this was not deemed significant. Indirectly these results could be used to ascertain the loading capacity of the column used, which if taken as the injection volume resulting in a 10% reduction in efficiency from the maximum efficiency obtained, would be equal to a maximum recommended injection volume of 0.6 μL .

Table 2
Dimensions and reported isocratic peak efficiencies of some organo-polymer monolithic columns in acetonitrile–water mobile phases at elevated temperatures and pressures.

Column	L, cm	I.D., mm	Flow rate/cross-section, cm/min	T_{max} , °C	P, bar	N theor. plates, m	Ref.
MS/BVPE poly(<i>p</i> -methylstyrene–1,2-bis(<i>p</i> -vinylphenyl)ethane)	8	0.2	32	50	80 (25 °C)	25,000–35,000	[41]
BVPE poly(1,2-bis(<i>p</i> -vinylphenyl)ethane)	20	0.2	32	25	n/a	49,000–70,000	[42]
ODMA/EDMA poly(<i>n</i> -octadecyl methacrylate–ethylene dimethacrylate)	100	0.25	6	80	15	20,000	[43]
Poly DVB poly(divinylbenzene)	23/42	0.25	20	200	n/a	6000 ^a	[35]
BuMA/EDMA poly(butyl methacrylate–ethylene dimethacrylate)	10	0.8	2	110	196	59,000	Present work

^a Calculated from chromatogram from Ref. [33] for a 23 cm column.

3.5.4. Separation of small molecules

Finally, a standard mixture of small organic molecules was separated on a prepared titanium housed monolithic column using an eluent of 60% acetonitrile–40% water and a column temperature of 110 °C. The obtained chromatogram is shown as Fig. 7(a). The prepared column was also used for the separation of the mixture of pesticides (Fig. 7(b)), but for comparative analysis of our column with previously published work (below), data was taken from Fig. 7(a). Clearly, the use of an applied gradient would improve the separation of small molecules, particularly the separation of the mix of nine pesticides shown. However, here the separation of pesticides was included simply to show the potential application of the new column to small organic molecules in RP-HPLC mode. For polymeric monolithic phases, this represents a real on-going challenge, and so although the separation could be improved with a gradient, it actually demonstrates more of the potential of the new monolithic phase by using just showing this isocratic separation.

The following peak efficiencies were achieved for uracil (~59,000 N/m), acetophenone (~52,000 N/m), benzene

(~52,000 N/m), toluene (~50,000 N/m) and naphthalene (~49,000 N/m). These results were compared to those obtained by Moravcova et al. [22] and Holdsvendova et al. [39], who utilised butyl methacrylate–*co*-ethylene dimethacrylate-based monolithic capillary columns and observed peak efficiencies up to ~34,000 N/m for benzene and ~29,000 N/m for toluene [22] and ~35,000 N/m for uracil, ~32,000 N/m for benzene and ~28,000 N/m for toluene [39]. Trojer et al. [41] tested MS/BVPE (*p*-methylstyrene/1,2-bis(*p*-vinylphenyl)ethane) monolithic capillary columns for the separation of small molecules under gradient conditions and elevated temperature, as well as under isocratic conditions and ambient temperature. For the latter conditions, efficiencies of up to 35,000 N/m were achieved. Greiderer et al. [42] reported efficiencies of up to 70,000 N/m for small molecules on a BVPE (poly(1,2-bis(*p*-vinylphenyl)ethane)) capillary column in isocratic conditions at ambient temperature (Table 2). Ueki et al. [43] studied the behavior of methacrylate-base monoliths, namely co-polymers of methacrylate esters with alkyl chains (C₂–C₁₈) and ethylene glycol dimethacrylate (EDMA). The best results were shown for the *n*-octadecyl methacrylate/EDMA monolithic stationary phases, where the efficiency of up to 20,000 N/m was achieved for the separation of 6 alkylbenzenes with elevated column temperatures (Table 2).

Therefore, in the work presented here with new titanium housed monoliths, the separation efficiencies are at least compa-

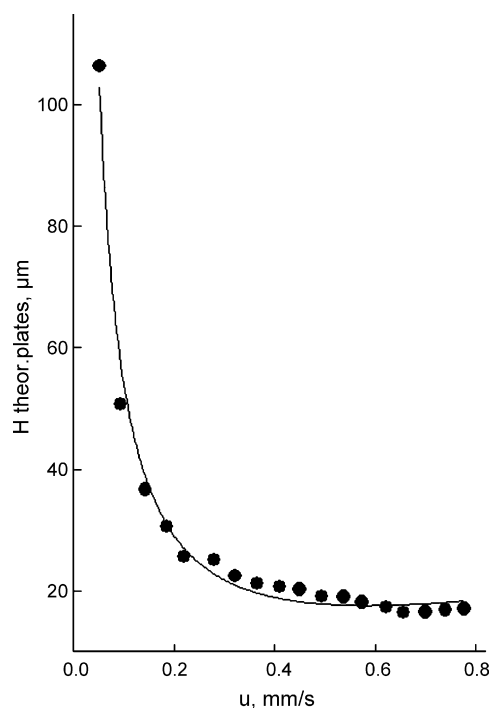


Fig. 5. van-Deemter plot for a titanium housed butyl methacrylate–ethylene dimethacrylate monolith, 100 mm × 0.8 mm I.D., mobile phase=90% ACN–10% water. Column temperature=100 °C. Sample: naphthalene (100 ppm, 0.2 μL injection volume). Data fitted using function $y = a + bx + c/x$ giving calculated coefficients $a = 4.25 (\pm 1.54) \times 10^{-3}$ mm, $b = 1.63 (\pm 0.42) \times 10^{-3}$ mm²/s and $c = 2.82 (\pm 0.08) \times 10^{-2}$ ms.

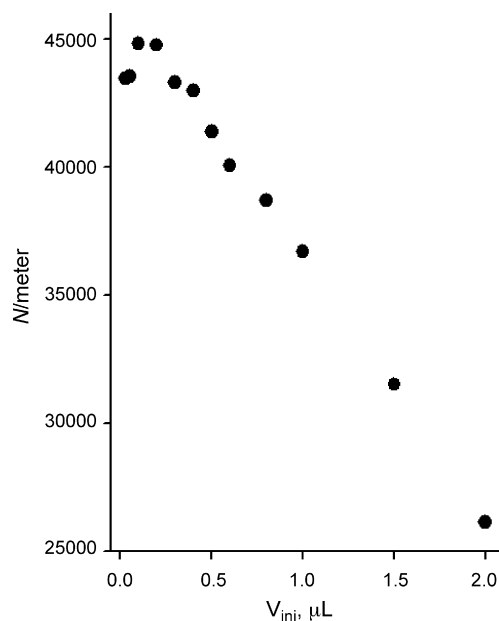


Fig. 6. The dependence of peak efficiency on the injection volume. Column: titanium housed butyl methacrylate–ethylene dimethacrylate monolith, 100 mm × 0.8 mm I.D., mobile phase=60% ACN–40% water. Analyte – naphthalene, concentration – 0.1 g/L.

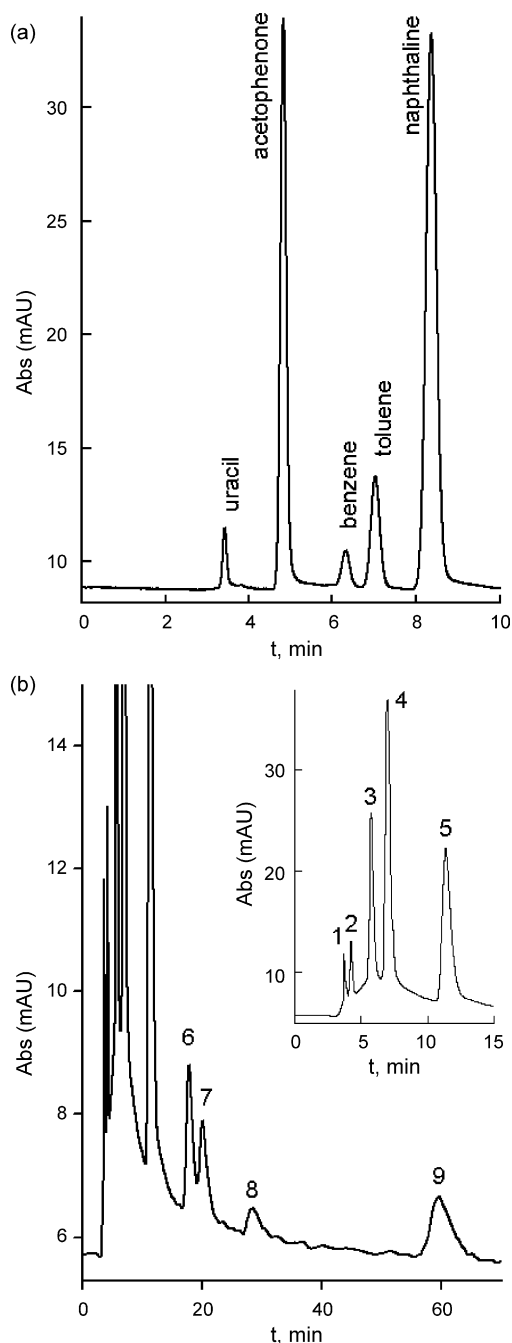


Fig. 7. Separation of a (a) test mixture on a titanium housed butyl methacrylate–ethylene dimethacrylate polymer monolithic stationary phase. Column dimensions: 100 mm \times 0.8 mm I.D. Mobile phase = 60% ACN–40% water, $F = 10 \mu\text{L}/\text{min}$, column temperature = 110 $^{\circ}\text{C}$, column backpressure = 19.6 MPa and (b) the separation of pesticides: 1 – Paraquat, 2 – Aldicarb, 3 – 2-hydroxy-4-methoxy-benzene, 4 – 5-chlorosalicylaldehyde, 5 – naphthalene, 6 – Chlorpyrifos, 7 – hexamethylbenzene, 8 – Dieldrin, 9 – DDT. Column: butyl methacrylate–ethylene dimethacrylate polymer monolith, 100 mm \times 0.8 mm I.D. Mobile phase = 60% ACN–40% water, $F = 10 \mu\text{L}/\text{min}$, column temperature = 70 $^{\circ}\text{C}$. UV detection at 254 nm.

able to previously published work, and in some cases significantly exceed previously published performance.

4. Conclusions

The results presented herein demonstrate a novel approach to the preparation of larger scale polymer monolithic station-

ary phases covalently bound to the titanium housing. It was shown that columns prepared according to this procedure exhibited high efficiency and can be utilised for the separation of low molecular weight molecules. The reproducibility studies showed that prepared columns can be used at high temperatures and pressures and are stable under these conditions with no evidence for monolith detachment from the titanium column housing.

The other important advantage of titanium housing is a better heat dissipation not only during synthesis but also for chromatographic separation at elevated temperature, which is extremely important for some separations where reasonable selectivity can be achieved with applied column temperature gradients [44].

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