



Polymerisation and surface modification of methacrylate monoliths in polyimide channels and polyimide coated capillaries using 660 nm light emitting diodes

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ABSTRACT

An investigation into the preparation of monolithic separation media utilising a cyanine dye sensitiser/triphenylbutylborate/*N*-methoxy-4-phenylpyridinium tetrafluoroborate initiating system activated by 660 nm light emitting diodes is reported. The work demonstrates multiple uses of red-light initiated polymerisation in the preparation of monolithic stationary phases within polyimide and polyimide coated channels and the modification of monolithic materials with molecules which absorb strongly in the UV region. This initiator complex was used to synthesise poly(butyl methacrylate-*co*-ethylene dimethacrylate) and poly(methyl methacrylate-*co*-ethylene dimethacrylate) monolithic stationary phases in polyimide coated fused silica capillaries of varying internal diameters, as well as within polyimide micro-fluidic chips. The repeatability of the preparation procedure and resultant monolithic structure was demonstrated with a batch of poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths in 100 μm i.d. polyimide coated fused silica capillary, which were applied to the separation of a model protein mixture (ribonuclease A, cytochrome C, myoglobin and ovalbumin). Taking an average from 12 chromatograms originating from each batch, the maximum relative standard deviation of the retention factor (k) for the protein separations was recorded as 0.53%, the maximum variance for the selectivity factor (α) was 0.40% while the maximum relative standard deviation in peak resolution was 8.72%. All maxima were recorded for the Ribonuclease A/Cytochrome C peaks.

Scanning electron microscopy confirmed the success of experiments in which poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths were prepared using the same initiation approach in capillary and micro-fluidic chips, respectively. The initiating system was also applied to the photo-initiated grafting of a chromophoric monomer onto poly(butyl methacrylate-*co*-ethylene dimethacrylate) monoliths within poly(tetrafluoroethylene) coated fused silica capillaries.

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1. Introduction

From the first report of photo-initiated synthesis of monolithic separation media by Viklund et al. [1] in 1997, UV light as the initiating source remains the state of the art for the production of controlled lengths of polymeric monolith within UV transparent moulds. Despite the popularity of this technique and the advances in light sources over the years, from mercury lamps [1], to fluorescent lamps [2], to light emitting diodes (LEDs) [3], there remains two major disadvantages to this approach. Firstly, the mould must be UV transparent to allow UV light to pass into the cavity to initiate polymerisation, which excludes the use of the standard and most durable polyimide coated capillaries. Secondly, the monomers and

porogens either must not absorb UV light or must absorb at a wavelength removed from the λ_{\max} of absorbance of the initiator, excluding the use of strong UV absorbers such as styrenes.

Several important advances in chromatographic technology have brought the issue of mould transparency to the foreground. The first of these is the recent introduction of the Agilent HPLC Chip, which employs specifically designed micro-fluidic chips fabricated from laser ablated laminated polyimide layers [4]. The second is the growing use of monolithic separation media in gas chromatographic applications, where polyimide coated capillaries are commonly used [5,6]. These show that there is a real need for a method of photo-initiated polymerisation which can be used with polyimide and polyimide coated moulds. While thermally initiated polymerisation is capable of producing monolithic materials within polyimide moulds the disadvantages of this method are that the polymerisations are generally rather long, in the region of 20 h, and there is no spatial control over the location of the monolith in the capillary [1,7]. UV initiated polymerisation allows spatial control of the monolith formation and reduced polymerisation times but cannot be used with polyimide coated capillaries due to absorption of the UV light by the polyimide coating. Visible light initiated polymerisation in the red region is the ideal solution to the issues arising from both of these methods, it too gives control over the location of the polymer in the capillary but without the need to remove the polyimide layer and average polymerisation times are far shorter than those recorded for thermally initiated polymerisations [8]. Additionally, as with UV initiated polymerisation, the polymerisations can be carried out at room temperature and there is the possibility for post-synthesis functionalisation of the monolith surface in a well-defined area using the same initiating system.

Dulay et al. [9] made the first advances in this area by presenting the visible light initiated synthesis of a sol-gel monolith within polyimide coated fused silica capillaries, performed using a cool fluorescent lamp emitting at 470 nm.

More recently, the authors of this present work were successful in further shifting the wavelength of the initiating light source into the red region, showing the first preparation of poly(glycidyl methacrylate-co-ethylene dimethacrylate) [poly(GMA-co-EDMA)] monoliths within polyimide coated fused silica capillaries [8]. In this case the reaction was initiated using a novel three component initiator system consisting of a cyanine dye/borate salt complex (3-butyl-2-[5-(1,3-dihydro-3,3-dimethyl-1-propyl-2H-indolylidene)-penta-1,3-dienyl]-1,1-dimethyl-1H-benzo[e]indolium triphenyl butyl borate, HNB660) and a secondary co-initiator (*N*-methoxy-4-phenylpyridinium tetrafluoroborate, MPPB) using a diode emitting at 660 nm [see electronic supplementary information (ESI) for structures].

This current work presents the full investigation of the novel initiating system and its utilisation to prepare organic polymer monoliths within polyimide coated fused silica capillaries and polyimide moulds. The synthesis of both poly(butyl methacrylate-co-ethylene dimethacrylate) [poly(BuMA-co-EDMA)] and poly(methyl methacrylate-co-ethylene dimethacrylate) [poly(MMA-co-EDMA)] monoliths in capillaries is demonstrated and the application of the poly(BuMA-co-EDMA) monoliths to the separation of a model protein mixture is shown, confirming that the monoliths produced using this method have porous structures and rigidity comparable to those prepared *via* the standard UV light initiated method and can be applied to chromatographic separations. Additionally, some specific and novel examples of the potential of this method for the synthesis and modification of monolithic separation media are also shown, (i) within polyimide micro-fluidic chips, and (ii) the application of the initiator system to facilitate the photo-initiated grafting of UV absorbing monomers on pre-existing monolithic scaffolds. The above applications of this new technology show the versatility of this approach and bring common polyimide moulds into the

range of moulds in which photo-initiated monolith synthesis can be carried out, which until recently [8,9] was not possible.

2. Experimental

2.1. Reagents

Acetic acid (ACS reagent grade, 99.7%), acetonitrile (HPLC grade, 99.9%), butyl methacrylate (BuMA, 99%), cytochrome C from bovine heart, 1-decanol (99%), ethylene dimethacrylate (EDMA, 98%), ethanol (spectrophotometric grade), formic acid (ACS reagent grade, 88%), methanol (HPLC grade, 99.9%), methyl methacrylate (MMA, 98%), myoglobin from equine skeletal muscle, *N*-methoxy-4-phenylpyridinium tetrafluoroborate (97%), ovalbumin from chicken egg white, 1-propanol (HPLC grade, $\geq 99.9\%$), ribonuclease A from bovine pancreas and 3-(trimethoxysilyl)-propyl methacrylate (TMSPM, 98%) were all purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA and Wicklow, Ireland). All chemicals were used as received with the exception of BuMA, MMA and EDMA, which were purified before use by passing over a bed of basic aluminium ($\sim 58 \text{ \AA}$) to remove inhibitors. 3-Butyl-2-[5-(1,3-dihydro-3,3-dimethyl-1-propyl-2H-indolylidene)-penta-1,3-dienyl]-1,1-dimethyl-1H-benzo[e]indolium triphenyl butyl borate (HNB660) was purchased from Spectra Group Limited, Inc. (Millbury, OH, USA) and used as received. A monomeric spiro pyran presenting a free vinyl terminal chain, 1'-(9-decenyl)-3',3'-dimethyl-6-nitrospiro[2H-1]-benzopyran-2,2'-indoline (SPM), was synthesised from a procedure described by McCoy et al. [10], which is outlined in the ESI. Water used in these experiments was obtained from a Milli-Q Ultrapure water filtration system from Millipore (Billarica, MA, USA).

2.2. Materials

Light emitting diodes (LEDs) were obtained from Roithner Laser Technik GmbH (Vienna, Austria) ($\lambda_{\max} = 660 \text{ nm}$, optical power = 3.5 cd at 20 mA) and MCD Electronics Ltd. (Albuquerque, NM, USA) ($\lambda_{\max} = 660 \text{ nm}$, optical power = 0.5 cd at 20 mA). Stripboard for fabrication of the LED arrays (shown in the ESI) and commercial power supply units operated in a constant voltage mode to power the LEDs were purchased from Maplins Electronics (Rotherham, UK). A small commercially available motor with a variable speed of rotation (Peats Electronics, Dublin, Ireland) was used at low speeds (ca. 34 rpm) for the polymerisation of standard poly(BuMA-co-EDMA) monoliths in capillary and for photo-initiated grafting. A second in-house fabricated motor, rotating at higher speeds of ca. 120 rpm, was used for the synthesis of standard poly(MMA-co-EDMA) using the LED array.

50–100 μm i.d. polyimide and 100 μm i.d. PTFE coated fused silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA). Polyimide micro-fluidic chips, with an internal channel 0.2 mm \times 0.2 mm \times 68 mm, were generously provided by Agilent Technologies (Santa Clara, CA, USA).

2.3. Instrumentation

Spectra of polyimide sheets were taken with a Cary 50 UV-vis spectrometer from Varian Inc., (Palo Alto, CA, USA). Optical micrographs of monolith filled micro-fluidic chips were taken with a TE200 optical microscope from Nikon (Tokyo, Japan) while filled capillaries were imaged using an EMZ-8TR video microscope (Meiji Techno, Saitama, Japan). Scanning electron micrographs of all poly(MMA-co-EDMA) monoliths were taken with an S-3400N Variable Pressure Scanning Electron Microscope from Hitachi (Tokyo, Japan) while poly(BuMA-co-EDMA) filled capillaries and chips were

Table 1
Experimental conditions for the preparation of full poly(BuMA-co-EDMA) monoliths in capillary and chip channels and full and PLOT poly(MMA-co-EDMA) monoliths in capillary channels.

Parameter	Poly(BuMA-co-EDMA) monoliths in capillary	Poly(BuMA-co-EDMA) monoliths in chip	Poly(MMA-co-EDMA) monoliths in capillary
Quantity of monomers	0.26 mmol BuMA (21 vol.%) 0.15 mmol EDMA (14 vol.%)	0.26 mmol BuMA (21 vol.%) 0.15 mmol EDMA (14 vol.%)	0.45 mmol MMA (24 vol.%) 0.17 mmol EDMA (16 vol.%)
Quantity of porogens	11.5 vol.% acetonitrile 23 vol.% 1-propanol 30.5 vol.% 1-decanol	11.5 vol.% acetonitrile 23 vol.% 1-propanol 30.5 vol.% 1-decanol	11 vol.% acetonitrile 21 vol.% 1-propanol 28 vol.% 1-decanol
Quantity of HNB660	0.25 mg (0.375% per wt monomers, pwm)	0.25 mg (0.375% pwm)	0.2 mg (0.25% pwm)
Quantity of MPPB	2.5 mg (3.75% pwm)	2.5 mg (3.75% pwm)	2 mg (2.5% pwm)
Total % of monomers	35%	35%	40%
Channel i.d.	100 μm	200 μm^2	100, 75, 50 μm
Number of 660 nm LEDs	1 (3.5 cd)	2 (3.5 cd each)	5 (LED array, 0.5 cd each)
Polymerisation time	2 h	4–6 h	1.5 h (100, 75), 1 h (50)
Rotation during polymerisation	Yes, 34 rpm	No	Yes, 120 rpm

analysed with an Ultra-55 analytical Scanning Electron Microscope from Carl Zeiss (Jena, Germany).

A TraceDec, on-column capacitively coupled contactless conductivity detector (C^4D) for scanning C^4D profiling, with a 375 μm capillary sensor head was purchased from Innovative Sensor Technologies (Vienna, Austria). Low pressure infusion pumps were purchased from KD Scientific (Holliston, MA, USA).

Capillary separations and back-pressure measurements were carried out using an Ultimate 3000 nano-HPLC (Dionex Corporation, Sunnyvale, CA, USA).

The switching of the grafted spiropyran was verified by reflective absorbance spectroscopy (RAS). A purposely designed holder fabricated in black acrylonitrile-butadiene-styrene (ABS) copolymer was constructed for this purpose as described in a previous paper by Scarmagnani et al. [11]. Reflectance UV-vis spectra were recorded using a S2000[®] spectrometer combined with a FCR-7UV200-2 reflection probe (7 $\mu\text{m} \times 200 \mu\text{m}$ cores) which was connected to a DH-2000-FSH, deuterium (215–400 nm) and halogen (400–1700 nm) light source, using a PTFE-based reflectance standard WS-1-SL to standardise measurements at 100% reflectance [12,13]. All spectrometric instrumentation was purchased from Ocean Optics Inc. (Eerbeek, Netherlands).

2.4. Procedures

2.4.1. Pre-treatment of the polyimide and PTFE coated fused silica capillaries

Before polymerisation within the fused silica capillaries, the channel walls were treated to ensure covalent attachment of the monolith using a procedure described by Křenková et al. [14], which is outlined in more detail in the ESI.

2.4.2. Pre-treatment of the polyimide micro-fluidic chips

The channels of the chip were flushed with acetone and dried under nitrogen flow, then flushed with water and dried under air, flushed with methanol and dried under nitrogen, before finally filling with the pre-polymer solution. No specific pre-treatment of the channel walls was necessary due to the polymeric nature of the polyimide.

2.4.3. Preparation of the pre-polymer solutions

All conditions for the preparation of pre-polymer solutions and synthesis conditions for the preparation of monoliths in capillary and micro-fluidic chips are outlined in Table 1.

In all cases, once the reagents were combined in the correct ratios in an amber vial, the pre-polymer solution was sonicated for 30 min to remove dissolved oxygen and ensure complete dissolution of the solid reagents. Amber vials were used to ensure the

pre-polymer solution had minimal interference from ambient light during preparation.

2.4.4. Synthesis of monoliths

The channels of the pre-treated capillaries and chips were filled with the pre-polymer solution (more specific details can be found within the corresponding ESI). The channels were then sealed using rubber septa and placed under the 660 nm LEDs or LED arrays for a specified length of time and with/without rotation as specified in Table 1. Once again all polymerisations were carried out in the dark to avoid interference from ambient light. Polymerisations were carried out at room temperature and, due to the negligible heat generation from LEDs [2], no heat was input into the system from the light source and no special precautions were necessary to keep the system cool. In some cases simple photo masking with black vinyl tape was carried out to show that the formation of the polymer could be localised within the channels. On completion of the polymerisation reaction the channels were flushed with acetonitrile to remove any unreacted reagents. The monoliths in capillaries and chips were then stored in water.

2.4.5. Synthesis of poly(BuMA-co-EDMA) in PTFE coated capillaries at 254 nm and the photoinitiated grafting of a monomeric spiropyran on the pore surface at 660 nm

A pre-polymer solution consisting of 36 μl (0.23 mmol) BuMA, 24 μl (0.13 mmol) EDMA, 47 μl cyclohexanol and 93 μl 1-decanol was added to a vial containing 0.6 mg 2,2-dimethoxyphenyl acetophenone (DMPAP). The solution was sonicated for 10 min and purged with nitrogen before filling into pre-treated PTFE coated capillaries by capillary action and sealing with rubber septa. The capillaries were placed in the cavity of an X1-1000 UV cross-linker to carry out the polymerisation. Each capillary was given an irradiation dose of 3 J/cm². After polymerisation the capillaries were flushed with methanol and then nitrogen, ready for further surface modification. The monomeric spiropyran, SPM, containing a long carbon chain with a vinyl group on the indoline ring, which is suitable for subsequent copolymerisation with divinylbenzene, was synthesised following a procedure described by McCoy et al. [10], as outlined in our previous work [15] (see ESI).

The spiropyran monomer, 1 wt.% (1 mg/ml) was weighed into an amber vial along with 0.02 wt.% (0.2 mg/ml) HNB660 and 2 mg MPPB. The monomer and initiators were dissolved in 180 μl acetonitrile, 360 μl 1-propanol and 460 μl 1-decanol. Finally, the mixture was sonicated for 1 h to ensure dissolution of all reagents and to remove dissolved oxygen in the mixture. The mixture was flushed through a poly(BuMA-co-EDMA) monolith in PTFE coated fused silica capillary and the ends were sealed with rubber septa as before. The capillary monolith was then connected to a motor and

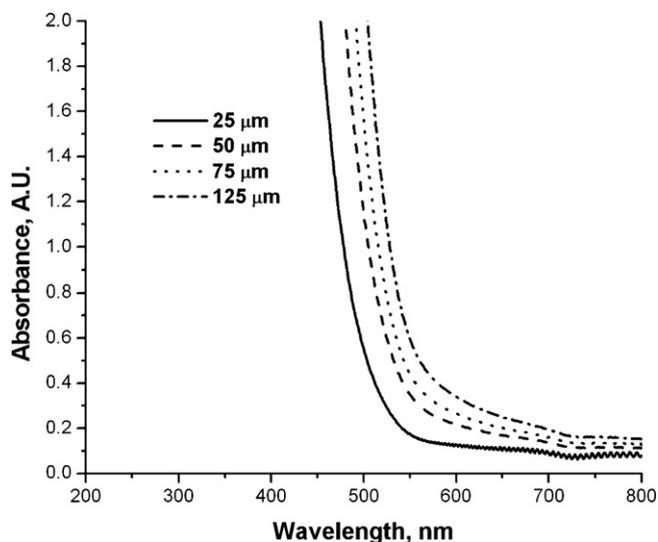


Fig. 1. Absorbance spectra of polyimide sheets of varying thickness; 25, 50, 75 and 125 μm .

rotated at 34 rpm while irradiated with 660 nm light for 2 h. After irradiation the monolith was flushed with ethanol to remove any unreacted reagents and any unbound spiropyran monomer from the monolith pores.

3. Results and discussion

3.1. Monolithic capillary columns

3.1.1. Optimisation of the polymerisation conditions

In our previous work [8] the absorbance spectrum of 375 μm o.d./100 μm i.d. polyimide coated fused silica capillary was measured using an Agilent 3D CE instrument, in order to demonstrate how the polyimide coating of the capillary only begins to allow the transmission of light when the λ_{max} is above 550 nm [8]. However, due to the limitations of the instrumentation it was not possible to measure the transmission through the capillary above 600 nm. To obtain a better estimation of the transmission in this region, the absorbance spectra of sheets of polyimide film of varying thicknesses (25, 50, 75, and 125 μm) were measured using a conventional UV–vis spectrometer. As the actual composition of the polyimide coating on Polymicro polyimide coated fused silica capillaries is not public information, sheets of KAPTON[®] polyimide (DuPont, Wilmington, DE, USA) were used as substitutes. Fig. 1 shows the absorbance spectra of the four films in the range 200–800 nm. The data from these absorbance spectra were then used to estimate the transmission of light through a polyimide film 20 μm thick, such as that used to coat the fused silica capillaries. It is clear from the absorbance spectra (Fig. 1) that an initiator absorbing in the region 600–800 nm would be the most suited to achieve photo-initiated polymerisation while irradiating through such a coating. Two commercially available dye sensitiser/initiator complexes are available within this region; one absorbing at 660 nm (3-butyl-2-[5-(1,3-dihydro-3,3-dimethyl-1-propyl-2H-indolylidene)-penta-1,3-dienyl]-1,1-dimethyl-1H-benzo[e]-indolium triphenyl butyl borate, (HNB660)), and a second at 780 nm (3H-indolium,2-[2-[2-chloro-3-[(1,3-dihydro-1,3,3-trimethyl-2H-indol-2-ylidene)ethylidene]-1-cyclohexen-1-yl]ethenyl]-1,3,3-trimethyl butyltriphenylborate, (HNB780)). Test experiments combining these initiator complexes with the *N*-methoxy-4-phenylpyridinium tetrafluoroborate (MPPB), monomers and porogens, and irradiating with an LED whose λ_{max} of emission is matched to the λ_{max} of absorbance of the initiator,

showed that the initiator absorbing at 660 nm, HNB660, was most suitable, with a more significant amount of polymer formation than the initiator absorbing at 780 nm, HNB780, within the same time period. Light transmission was found to be ca. 80% at 660 nm (calculations in ESI), with the lower wavelength LED also providing the higher light energy, meaning more photons can be absorbed by the sensitiser. As these initiators work *via* a mechanism known as photo-induced electron transfer, increasing the number of photons of energy imparted to the system increases the number of electrons which can be transferred between components of the system [16]. This in turn will increase the potential number of radical species generated leading to a more efficient polymerisation reaction, which needs less time to reach completion.

From previous experiments [8] it was known that a single 660 nm LED, along with 0.25% of the dye sensitiser/borate salt complex (HNB660) per weight of monomers (pwm) and 2.5% of the *N*-methoxy-4-phenylpyridinium tetrafluoroborate (MPPB) pwm was sufficient to initiate the polymerisation of GMA and EDMA in the presence of acetonitrile, 1-propanol and 1-decanol as porogens. In this current work the monomers used have slightly lower observed reaction rates than the GMA, although the cross-linker remains the same, and in the case of the micro-fluidic chip, the layer of polyimide is increased to 150 μm reducing light transmission into the channel to ca. 55% (Calculations in ESI). Due to these differences, the polymerisation conditions were modified in order to ensure the quantity of initiator and the number of LEDs used was sufficient to initiate the polymerisation.

Determining the correct percentage of HNB660 and MPPB necessary to initiate the polymerisation was done by simple single parameter optimisation. In the case of the MMA/EDMA pre-polymer solution, 0.25% HNB660 and 2.5% MPPB were found to be sufficient to initiate the polymerisation, with an LED array consisting of 5 LEDs of intensity 0.5 cd each used as the initiating source. Together these provided monoliths of desired thickness and length, i.e. that the capillary was completely filled with polymer, within 1–1.5 h. The concentration of initiators in the BuMA/EDMA pre-polymer solution were increased to 0.375% HNB660 pwm and 3.75% MPPB pwm and polymerisation was activated using one single 660 nm LED with an intensity of 3.5 cd, which produced the required polymer thickness in 2 h. For the polymerisation in chip, two LEDs were used and the polymerisation time was increased to 4 h to achieve the desired structure.

Using the modified pre-polymer solutions and polymerisation reaction times, a batch of poly(BuMA-*co*-EDMA) monoliths were prepared in capillaries with an internal diameter of 100 μm . Poly(MMA-*co*-EDMA) monoliths were also prepared, however, in capillaries of varying internal diameters (100, 75, and 50 μm). Polymerisation time was reduced to 1 h for the poly(MMA-*co*-EDMA) monolith in 50 μm i.d. capillary as the monolith became too dense after 1.5 h of irradiation.

3.1.2. Characterisation of the monolithic materials

Scanning electron microscopy (SEM) was used to verify the porous architecture of the synthesised monoliths and ensure they were well attached to the channel walls, affording the stationary phase with good mechanical stability. Fig. 2 shows an image of each of the four different monoliths synthesised.

It is clear from these images that the globular porous structure, characteristic of monolithic polymers, has been achieved along with wall attachment. The globule size of the poly(BuMA-*co*-EDMA) monoliths was measured from SEM imaging as less than 1 μm , while those of the poly(MMA-*co*-EDMA) monolith were in the range of 1.5–2 μm . Smaller globule sizes in the poly(BuMA-*co*-EDMA) monoliths may be attributed to higher initiator concentration in the pre-polymer solution causing earlier phase separation.

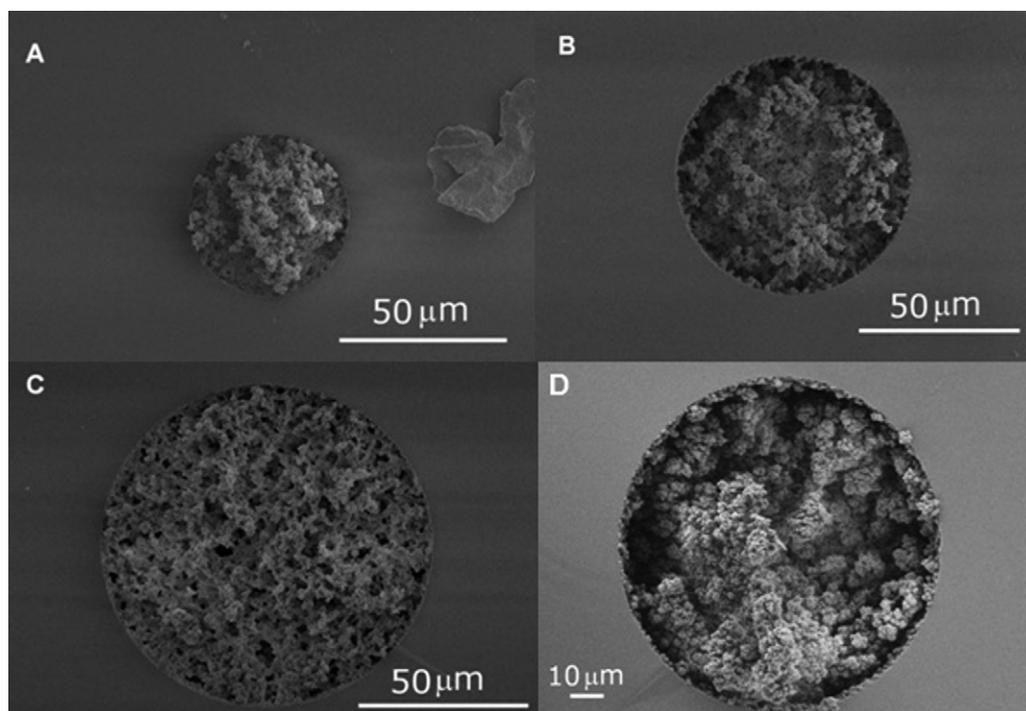


Fig. 2. Scanning electron micrographs of (a) poly(MMA-co-EDMA) in 50 μm i.d. capillary, (b) as (a) in 75 μm i.d. capillary, (c) as (a) in 100 μm i.d. capillary, and (d) poly(BuMA-co-EDMA) in 100 μm i.d. capillary.

Optical microscopy was also used to detect the presence of wall voids in the capillaries and verify that the polymer formation could be localised using photo-masking. The obtained images (not shown here) are presented in the ESI and confirm that no voids are present. They also show that a simple photo-masking approach can spatially control the location of the polymer within the channel.

With decreasing globule and pore size the back pressure of the monolith will increase, therefore for a monolith with relatively small pores such as the poly(BuMA-co-EDMA) monolith shown in Fig. 2(a) the back pressure would be expected to be relatively high. This is confirmed by back pressure data (not shown in text, presented in ESI), which shows the effect of flow rate on back pressure per unit of length for poly(BuMA-co-EDMA) monoliths formed within 100 μm i.d. capillaries.

The back pressure of the poly(BuMA-co-EDMA) monolith at 1 $\mu\text{l}/\text{min}$ was already 1.2 MPa/cm, however, the back pressure of the poly(MMA-co-EDMA) monolith in the same diameter capillary was almost 10 times lower, at just 0.18 MPa/cm (data in ESI). Comparing these two monoliths it is clear that the significant difference in pressure depends on globule size. While a large difference exists in the permeability of these two different types of polymers, this can be tailored by modifying monomer concentration and adjusting polymerisation time as necessary as described by Ratautaitė et al. [17].

Finally, lateral structural homogeneity of the capillary housed monoliths was investigated using scanning capacitively coupled contactless conductivity detection (sC⁴D). sC⁴D provides a profile of the monolith along the length of the column, which non-invasively confirms structural homogeneity and identifies any significant zones of irregularity. This procedure was first reported by Gillespie et al. [18] and is described in detail in the ESI. The relative standard deviation between the data points is indicative of the degree of homogeneity of the polymer filling [18–20]. This is an important characterisation when LED arrays are involved in the preparation of the monolith, as there is generally a spacing of a few mm between each LED and each LED may not emit the exact same light intensity as its neighbour, thus patches of increased/decreased density

along the length of the column can result. This can be rectified by the rotation of the capillary during polymerisation but if not corrected it can have significant effects on the homogeneity of the column, as shown in our previous work [21]. Fig. 3 shows the lateral conductivity profile of the three different poly(MMA-co-EDMA) batches ($n=3$ in each batch), as these were produced using LED arrays as the initiating light source.

It is clearly seen from Fig. 3 that monoliths produced with the LED array which are rotated during polymerisation do not appear to suffer from inhomogeneities along their length. The relative standard deviation for each of the batches was 4.62%, 3.23% and 5.83%

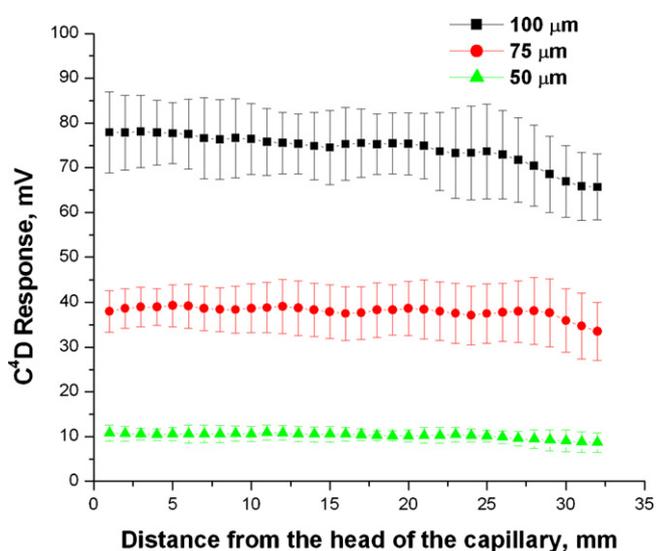


Fig. 3. Scanning C⁴D profiles showing the distance from the head of the column (mm) vs. the C⁴D response of batches ($n=3$ in each batch) of poly(MMA-co-EDMA) monoliths formed within 50 (triangle), 75 (circle) and 100 (rectangle) μm i.d. polyimide coated fused silica capillaries. Error bars show standard deviation. (For interpretation of the references to color in this sentence, the reader is referred to the web version of the article.)

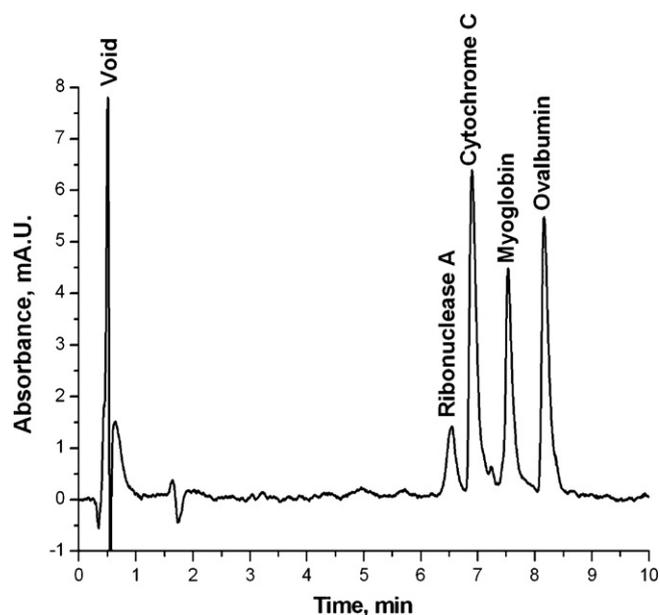


Fig. 4. Example of a separation of a model protein mixture (ribonuclease A, cytochrome C, myoglobin and ovalbumin) on a poly(BuMA-co-EDMA) monolith, of average length 6 cm and inner diameter 100 μm synthesised by red light initiated polymerisation within the channel of a polyimide coated fused silica capillary. Separation conditions: 1 $\mu\text{l}/\text{min}$ flow rate; 50 μl injection volume; 0.1 mg/ml sample concentration; gradient elution 0–60% B in 5 min where B is 0.1% FA in ACN and A is 0.1% FA in water; UV detection at 210 nm.

for the 100, 75 and 50 μm i.d. capillaries, respectively. The low RSD value also indicates that there is little variance in the product of the polymerisation reaction and that the method is highly reproducible.

3.1.3. Chromatographic applications

While characterisation is extremely important, only application of the monolithic polymers for the separation of test analytes will give an indication of their chromatographic performance and, in this context, the success of the polymerisation method. Here the separation of a 0.1 mg/ml model protein mixture containing ribonuclease A, cytochrome C, myoglobin and ovalbumin was performed on a full poly(BuMA-co-EDMA) monolith within the channel of a 100 μm i.d. polyimide coated fused silica capillary, to verify the quality of the stationary phase and of the initiation method. The separation and separation conditions are shown in Fig. 4.

It is clear from Fig. 4 that the proteins are well separated with sharp, narrow peaks and good resolution. The elution order of the peaks in Fig. 4 correlates very well with that presented by Nischang et al. [22], who have performed the separation of the same mixture of proteins on poly(BuMA-co-EDMA) monoliths synthesised by thermally initiated polymerisation. This indicates that red light initiated polymerisation is a robust method and can produce monoliths with similar structural characteristics, and hence chromatographic performance, to those synthesised by alternative methods.

Each separation was run three times on four different columns giving a total of 12 chromatograms. Retention factors (k), selectivity (α) and peak resolution (R_s) were calculated from each chromatogram and the data is presented in Table 2. It can be seen from the results presented in this table that this model separation is repeatable both between runs and between columns, with a maximum variance in k of 0.53% (Cytochrome C peaks), in α of 0.40% (Cytochrome C/Ribonuclease A peaks) and in resolution of 8.72% (Ribonuclease A/Cytochrome C peaks).

3.2. Monoliths within micro-fluidic chips

In the previous sections it has been shown that this initiating technique can be used to synthesise monoliths of different lengths and thicknesses within capillaries of varying internal diameters rapidly and easily using a cyanine dye sensitizer/borate salt/alkoxyphenylpyridinium salt initiating complex and a number of 660 nm LEDs. From here the next logical step was to investigate the applicability of this technique to different types of moulds, in particular micro-fluidic chips. To study this, polyimide micro-fluidic chips with channel dimensions of 0.2 mm \times 0.2 mm \times 68 mm were used. These chips were fabricated entirely from layers of polyimide and had no other chemical modifications or coatings. The top layer of polyimide above the channel was 150 μm thick, compared to just 20 μm coating of the previously discussed fused silica capillaries, thus reducing light transmission from ca. 80% to 55%. To compensate for this, two 660 nm LEDs were employed and the polymerisation time was increased to 4–6 h. Although this time is longer than that described for the polymerisations in capillaries, it is still a significant improvement compared to the time necessary for the thermally initiated polymerisation carried out in the same type of chips by Levkin et al. [23]. In addition, photo-initiation again provides a means for much greater spatial control through the use of photo-patterning. An SEM image of the channel after the polymerisation reaction (Fig. 5 (left)), shows that these modifications to the polymerisation procedure allowed for the formation of monolithic polymers, which entirely filled the channels and which appeared to be well attached to the channel walls.

The monoliths produced in these formats also exhibited the desired globular porous structure characteristic of organic polymer monoliths, similar to that shown in Fig. 2. Further SEM analysis confirmed that the globule size was again in the range of 1–2 μm , the same as that observed for the full monolith in capillary. Optical micrographs of the channel (Fig. 5 (right)), show that there were no obvious voids along the channel walls, verifying good wall attachment. The sharp edges of the monolith within the channel also proved that the location of the monolith within the channel can easily be controlled with simple photo-masking.

As the monolithic structure and chemical composition is similar to that demonstrated within fused silica capillaries, these monoliths have clear potential to be applied to on-chip separations in the near future. These polyimide chip based monoliths are the subject of an ongoing study which will verify their applicability for use as an effective separation medium.

3.3. Photo-initiated grafting of chromophoric monomers using red light

Monomers which absorb strongly in the UV region are often used to form [24] or modify monolithic stationary phases. One method of introducing such monomers into the monolithic scaffold is by direct copolymerisation commonly done using thermal [25] or radiation [26] curing methods, although more recently this was carried out using visible light initiated polymerisation [21]. However, direct copolymerisation can limit the activity of the functional monomer as much of the reactive sites are trapped in the bulk. Grafting of monomers onto the surface of pre-existing monolithic scaffolds is a method of ensuring that the maximum amount of reactive sites are present at the surface. Grafting is generally initiated by irradiation with UV light causing problems when using UV absorbing monomers and therefore, UV absorbing monomers are generally bound to the surface of a monolithic stationary phase by chemical interaction with surface reactive groups. However, the approach described herein, also had obvious additional potential to be exploited for application within the area of photo-initiated surface grafting, with the developed visible light initiating system

Table 2
Retention factors (k), selectivity (α) and resolution of peaks (R_s) (with SD and % RSD) for each separation of model protein mixture run on poly(BuMA-co-EDMA) monolithic column synthesised by visible light initiated polymerisation within 100 μm i.d. polyimide coated fused silica capillary. Selectivity and peak resolution were calculated for each relative to the peak eluting immediately before.

Peak Ribonuclease A	K	α	R_s	Peak Cytochrome C	K	α	R_s
C1 R1	6.02	–	1.83	C1 R1	6.45	1.07	2.78
C1 R2	6.01	–	1.81	C1 R2	6.45	1.07	2.91
C1 R3	6.02	–	1.71	C1 R3	6.44	1.07	2.87
C2 R1	5.98	–	1.51	C2 R1	6.36	1.06	3.11
C2 R2	5.98	–	1.81	C2 R2	6.37	1.07	3.29
C2 R3	6.00	–	1.79	C2 R3	6.41	1.07	3.21
C3 R1	6.00	–	1.90	C3 R1	6.41	1.07	3.06
C3 R2	5.97	–	1.75	C3 R2	6.36	1.07	2.91
C3 R3	5.99	–	1.67	C3 R3	6.37	1.06	2.80
C4 R1	6.04	–	1.86	C4 R1	6.42	1.06	2.66
C4 R2	6.03	–	1.38	C4 R2	6.38	1.06	2.83
C4 R3	6.04	–	1.69	C4 R3	6.42	1.06	2.67
Batch average	6.01 \pm 0.02 (0.40% RSD)	–	1.72 \pm 0.15 (8.72% RSD)	Batch average	6.40 \pm 0.03 (0.53% RSD)	1.07 \pm 0.004 (0.40% RSD)	2.93 \pm 0.20 (6.92% RSD)
Peak Myoglobin	K	α	R_s	Peak Ovalbumin	K	α	R_s
C1 R1	7.03	1.09	2.99	C1 R1	7.68	1.09	–
C1 R2	7.03	1.09	3.11	C1 R2	7.69	1.09	–
C1 R3	7.03	1.09	3.16	C1 R3	7.70	1.10	–
C2 R1	6.95	1.09	2.73	C2 R1	7.61	1.09	–
C2 R2	6.96	1.09	2.80	C2 R2	7.62	1.09	–
C2 R3	7.02	1.10	2.77	C2 R3	7.72	1.10	–
C3 R1	7.01	1.09	2.81	C3 R1	7.66	1.09	–
C3 R2	6.96	1.09	2.75	C3 R2	7.62	1.09	–
C3 R3	6.96	1.09	2.71	C3 R3	7.62	1.09	–
C4 R1	7.02	1.09	2.86	C4 R1	7.66	1.09	–
C4 R2	7.02	1.10	2.86	C4 R2	7.66	1.09	–
C4 R3	6.99	1.09	2.87	C4 R3	7.64	1.09	–
Batch average	7.00 \pm 0.03 (0.46% RSD)	1.09 \pm 0.003 (0.27% RSD)	2.87 \pm 0.14 (5.09% RSD)	Batch average	7.66 \pm 0.04 (0.47% RSD)	1.09 \pm 0.002 (0.21% RSD)	–

C indicates column and R indicates run.

being used as an effective initiator for the photo-initiated grafting of UV absorbing monomers.

To demonstrate this potential application, a monomeric photo-switchable spiropyran dye, 1',3'-dimethyl-6-nitrospiro[2H-1]-benzopyran-2,2'-indoline (structure and synthesis are given in the ESI), synthesised for a previous work on photo-controllable electroosmotic pumps [15], was chosen as a test molecule due to its complete absorbance in the UV region and its considerable visible region absorbance. Fig. 6 shows the absorbance spectrum of the spiropyran monomer overlaid with the absorbance spectrum of the dye sensitiser and the emission spectrum of the 660 nm LED. It is clear from Fig. 6 that the spiropyran does not

compete with the sensitiser for light from the initiating source, having negligible absorbance above 600 nm, but would be completely incompatible with any initiation system requiring excitation below this wavelength.

The grafting procedure was carried out in a single step, with both monomers and initiators present in the monolith channels at the same time. Once grafting was terminated, after 2 h, the monolith was flushed copiously with ethanol to remove unbound spiropyran – the dye being very soluble in short chain alcohols. After washing the monolith retained a faint pink colour indicating that spiropyran remains bound to the scaffold. As this faint colour does not photograph well, the capillary was irradiated with

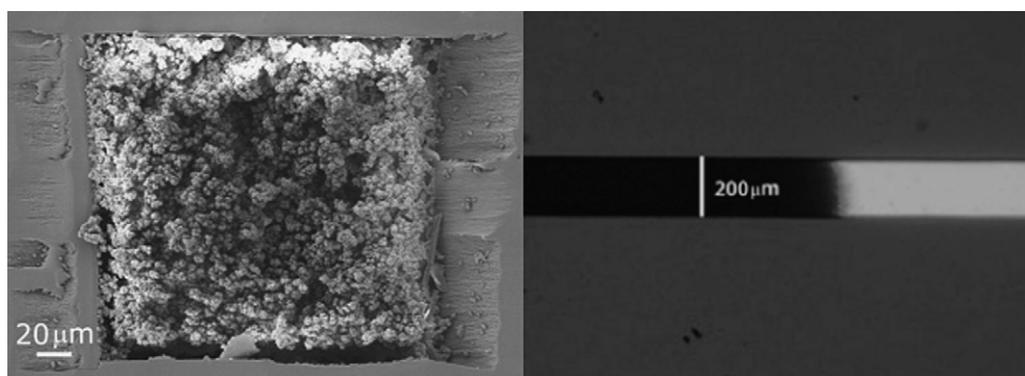


Fig. 5. (left) Scanning electron micrograph and (right) optical micrograph of a poly(BuMA-co-EDMA) monolith formed within the 200 μm \times 200 μm channel of a polyimide micro-fluidic chip using red light initiated polymerisation.

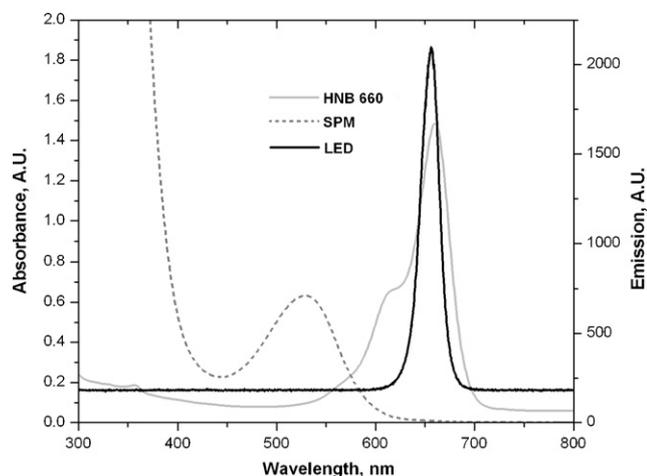


Fig. 6. Absorbance spectra of the spiropyran monomer (SPM) and the cyanine dye sensitizer (HNB660) overlaid with the emission spectrum of the 660 nm LED used to initiate the photo-grafting.

a 375 nm LED which causes the spiropyran to fluoresce [11], which is clearly seen in the inset of Fig. 7. As the spiropyran is a photo-switchable dye monomer, existing in both a colourless, uncharged form (spiropyran, SP) and an intensely coloured zwitterionic form (merocyanine, MC) depending on the wavelength of the irradiating light source [27,28], it was important to verify that the photo-switching property remains once the grafting is complete. This verification was done using reflective absorbance spectroscopy (RAS) and the results are shown in Fig. 7.

Due to the need to use RAS to determine the switching of the spiropyran it was necessary to carry out the grafting reaction on a monolith encased in PTFE coated capillary. It can be clearly seen in Fig. 7 that after 2 min of irradiation with UV light an absorbance maximum appears at approx. 540 nm corresponding with the λ_{max} of the spiropyran monomer in ethanol solution shown in Fig. 6. Irradiating with white light and measuring at several intervals during the irradiation, shows that as the irradiation time increase, the absorbance band decreases until at 3 min the maximum at 540 nm disappears. This confirms that both the photo-grafting of

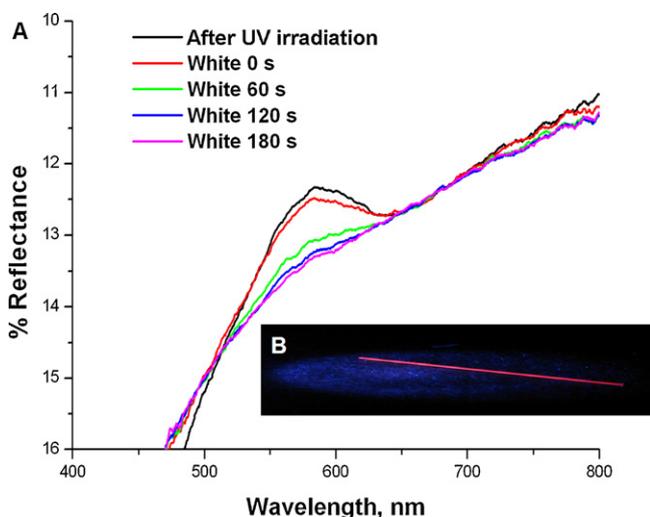


Fig. 7. (A) Reflective absorbance spectra of a spiropyran grafted poly(BuMA-co-EDMA) monolith in 100 μm PTFE coated fused silica capillary taken after 2 min irradiation with UV light and 0, 60, 120 and 180 s of irradiation with white light, inset (B) Photographic image of the spiropyran grafted poly(BuMA-co-EDMA) monolith in the zwitterionic merocyanine form fluorescing strongly under irradiation from a 375 nm LED.

the molecule with the 660 nm initiator system has been successful, and that the surface grafted molecule has maintained its desired photochromic activity.

4. Conclusions

Here the visible light initiated synthesis of methacrylate based monoliths within a variety of formats (polyimide coated fused silica capillaries of varying internal diameters, polyimide micro-fluidic chips, and the photo-initiated grafting of a UV absorbing monomer on the surface of a pre-existing monolithic scaffold) have been presented. These successful demonstrations of the newly developed red-light initiation system, show that the preparation of monolithic stationary phases for LC, GC and CEC applications are no longer limited to thermally initiated polymerisations within polyimide coated capillaries and UV light initiated polymerisations within UV transparent moulds. Photo-initiated polymerisation within polyimide coated capillaries has been shown to be a simple and relatively rapid method to achieve high quality methacrylate monolithic stationary phases and grafted stationary phases, with many potential applications. Further work will involve the tailoring of this method for the synthesis of styrenic monoliths within polyimide moulds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2011.03.021.

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