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Production of polymer monolithic capillary columns with integrated gold nano-particle modified segments for on-capillary extraction

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ABSTRACT

A number of polymer butyl methacrylate-*co*-ethylene dimethacrylate and lauryl methacrylate-*co*-ethylene glycol dimethacrylate monolithic phases within capillary columns were produced with integrated gold nano-particle (GNP) modified segments for on-capillary extraction and subsequent separation of biomolecules. Photo-masking and photo-grafting techniques were used to produce an aminated zone at the start of the polymer monolithic columns, which was then converted to a nano-agglomerated section using GNPs, followed by the otherwise unmodified monolith. The extent of amination was verified using scanning capacitively coupled contactless conductivity (sC^4D) and the C^4D profiles of modified monoliths monitored throughout the entire fabrication process, as a quality control technique. Two approaches to monolith amination were compared for achieving maximum GNP coverage, using surface photo-grafting of either vinyl azlactone or glycidyl methacrylate, prior to reaction with ethylenediamine. The modified monolithic columns were applied to the on-column trapping and subsequent reversed-phase separation of protein standards.

1. Introduction

The production of stationary phases for liquid chromatography exhibiting dual, sequential or gradient stationary phase functionality is subject to significant technical challenges. However, when available, such phases can offer new and potentially beneficial possibilities in many modes of chromatography, such as for example 'single-column' solid phase extraction and separation, 'on-column' sample preconcentration and focusing, 'on-column' sample pre-treatment and matrix removal (including selective removal of large biomolecules) or simply enhanced and targeted selectivity [1,2]. Using traditional particle packed columns, formation of the above multi-function columns can prove particularly difficult. For example, the packing of differing particle types within single column housings can cause problems in maintaining packing pressure whilst changing particle slurry mixtures, and the nature of the slurries themselves may vary considerably for different types of particle. Moreover, particle type compatibility with mobile phase conditions, such as pH, temperature or pressure, may too vary significantly and eventually lead to column failure in one or more of the segmented functional zones. The post-packing (non-invasive) characterisation of the stationary phase bed is also an additional challenge, as currently few methods exist to visualise boundaries between packed beds within non-transparent column housings.

On the other hand, the use of polymer monoliths for the production of gradient, segmented and sequential functionality columns has received considerable attention in recent years. The simplicity of monolith formation within various column housings, and its subsequent *in-situ* surface modification, is clearly an advantage to this approach and obviously eliminates any technical issues relating to column packing. A number of impressive studies have been reported demonstrating the application of polymer monolithic phases expressing gradient [3], dual [4] or multiple functionalities [5]. For example, Pucci et al. were amongst the first to investigate monolithic columns with a gradient of surface functionalities prepared via photo-initiated grafting and their application to separations using capillary electrochromatography [6]. More recently, Urbanova and Svec have also reported upon the development of a monolithic polymer layer with a gradient of hydrophobicity for the separation of peptides, and applied it to the two-dimensional thin layer chromatography and MALDI-TOF-MS detection [7].

The modification of polymer monoliths with nano-particles for various chromatographic and electrophoretic based applications is currently a much researched topic [8]. The common modification approaches used, either achieve incorporation of nano-particles via encapsulation within the monolith during polymerisation [9–11] or through post-polymerisation surface attachment [12,13]. The latter method, which has significant advantages in terms of surface coverage, has been successfully demonstrated using gold nano-particles (GNPs) based upon either amine [14,15] or thiol based [16–18] chemistries. For example, Connolly et al. [14] prepared poly(butyl methacrylate-*co*-ethylene dimethacrylate) (poly(BuMA-*co*-EDMA)) monoliths incorporating GNPs, investigating both thiol and amine immobilisation protocols. In this study, amine

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immobilisation resulted in a higher surface coverage of nano-particles compared to thiol immobilisation. In an extension of this work, Alwael et al. [15] immobilised similar GNPs onto EDMA based monoliths contained within polypropylene pipette tips, using the amine based immobilisation option. A carbohydrate binding protein (lectin) was immobilised onto the GNPs, and applied to the selective extraction of glycoproteins with terminal galactose. The extract was then washed from the pipette tip and further injected onto a reversed-phase column for separation.

Xu et al. [17] reported the fabrication of poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) (poly(GMA-*co*-EDMA)) monoliths bearing thiol groups. The columns were further modified with gold using two methods of nano-particle attachment, either through flushing the pre-formed nano-particles through the monolithic column, or by *in-situ* reduction of gold chloride and sodium citrate. The columns were used for the selective trapping of cysteine containing peptides, with subsequent separation on a commercially available reversed-phase Acclaim PepMap packed capillary column. In the latest work from this group, Lv et al. have reported an improved procedure leading to an increased surface loading of GNPs upon poly(GMA-*co*-EDMA) monolithic phases, up to 60 wt.% gold, using reaction of the base monolith with cystamine, followed by cleavage of the cystamine disulphide bond using tris(2-carboxylethyl)phosphine, liberating the two desired thiol groups for stable gold attachment [18].

Photo-grafting is an elegant surface modification technique, which has been used in the functionalisation of monolithic columns with numerous selectivities, upon morphologically pre-optimised monolithic structures [4–7,19–21]. The technique has several advantages over alternative methods, such as thermally initiated surface modification, not least of which is the ability to graft spatially isolated functionalised zones within capillary and micro-fluidic channel housed monoliths. The potential advantages of this type of photo-grafting approach to produce 'segmented' chemistry upon a single column are also numerous, but may specifically include the elimination of inter-column void volumes, simplicity of instrumental design and potential reduction in switching valves etc., potential benefits in recovery and advantages in system miniaturisation and transfer to micro-fluidic manifolds.

The following paper reports upon the production of dual functionality monoliths using a photo-grafting approach to the formation of a spatially isolated GNP functionalised region at the start of the capillary monolith for trapping of biomolecules, for either on-capillary sample clean-up, or for their concentration prior to release and separation upon a single monolithic capillary column. Two photo-grafted chemistries to achieve subsequent amination of the monolithic phase were compared, followed by their modification with 20 nm citrate stabilised pre-formed GNPs. The entire fabrication procedure for producing the dual function monolithic column, including the final modification with GNPs, was monitored using scanning capacitively coupled contactless conductivity detection (sC^4D) [22].

2. Materials and methods

2.1. Instrumentation

Photo-polymerisation of monoliths and all photo-grafting steps were performed in a Spectrolinker Crosslinker Model XL1000 (Spectronics, Westbury, NY, USA). A Harvard Apparatus PHD 2000 syringe pump (Harvard Apparatus, Holliston, MA, USA) was used to introduce monomer solutions into the pre-formed monoliths for photo-grafting. In all other instances, a Knauer Smartline 100 V 5010 pump (Knauer, Berlin, Germany) was used to pump through monoliths at flow rates ranging from 1 to 5 μL/min. A Mistral Column Oven 880 (Spark Holland, The Netherlands) was used for thermal modification of photo-grafted epoxy groups. A PEEK loop of approximately 300 μL, was filled with gold nano-particle solution, and was placed between the pump and the column, to introduce gold nano-particles to the column. A TraceDec capacitively coupled contactless conductivity (C^4D) detector (Innovative Sensor Technologies, GmbH, Innsbruck, Austria) was used for monolith characterisation with settings of 0 dB, 50% gain and 0 offset. A Hitachi S-5500 field emission scanning electron micrograph (FE-SEM) (Hitachi, Maidenhead, UK) used for imaging. For chromatographic studies, a Dionex Ultimate 3000 capillary LC system was used, with a split flow rate of 1:101, delivering a flow rate of 2 μL/min. Protein standards were prepared at 50 μg/mL for ribonuclease B, insulin, and bovine serum albumin. The proteins were injected onto the column via 120 nL loop. For separation, a hold up time of 5 min was applied, followed by a 10 min gradient from 100% A (5% ACN, 0.1% TFA) to 100% B (95% ACN, 0.1% TFA). Proteins were detected using UV detection at a wavelength of 214 nm.

2.2. Scanning capacitively coupled contactless conductivity (sC^4D)

Scanning capacitively coupled contactless conductivity (sC^4D) was performed as described previously [23]. Each monolith produced was equilibrated with water and profiled using sC^4D prior to surface modification procedures. Aminated monoliths were protonated using dilute HNO_3 solution for 1 h, and then flushed with water until the effluent reached a neutral pH, prior to further sC^4D profiling. Following the immobilisation of GNPs, the monolithic columns were again scanned in deionised water.

2.3. Materials and reagents

Butyl methacrylate (BuMA), lauryl methacrylate (LMA), ethylene glycol dimethacrylate (EDMA), glycidyl methacrylate (GMA), 2,2-dimethoxy-2-phenylacetophenone (DAP), benzophenone, 1,4-butanediol, 1-propanol, 3(trimethoxysilyl)-propyl methacrylate, ethylenediamine, nitric acid, sodium hydroxide, hydrochloric acid, gold(III) chloride trihydrate, and trisodium citrate were purchased from Sigma-Aldrich (Dublin, Ireland), and used as supplied.

Acetone and methanol were supplied by Labscan (Stillorgan, Dublin, Ireland). Vinyl azlactone (VAL) was purchased from TCI Europe (Boerenveldseweg, Belgium). Deionised water was provided by a MilliQ Direct Q5 water purification system from Millipore (Millipore Bedford, MA, USA). PTFE-coated fused silica capillary (100 μm i.d., 360 μm o.d.) was purchased from Composite Metal Services (Shipley, West Yorkshire, UK).

2.4. Fabrication of butyl methacrylate columns

Fused silica capillary was vinylised using a procedure described previously [24]. A total of ten monolithic columns consisting of BuMA-*co*-EDMA, were prepared for the optimisation of grafting procedures. Each monolith was prepared using a 40% monomer mixture (24% BuMA, 16% EDMA) in the presence of 60% decanol. Photo-polymerisation was achieved using 2 J/cm^2 UV irradiation. Three columns, named hereafter BuMA-V1, BuMA-V2, and BuMA-V3 were fabricated, for subsequent VAL grafting. To investigate the effect of varying VAL concentration during photo-grafting, two more columns were produced, named BuMA-V30 and BuMA-V40 (see Table S1). Columns BuMA-G1, BuMA-G2 and BuMA-G3 were fabricated for subsequent GMA grafting. The final columns measured 100 mm in length (for grafting optimisation). Following fabrication the monolithic columns were subjected to sC^4D profiling as per Section 2.2.

2.5. Fabrication of lauryl methacrylate columns

In total, five columns named LMA-G1, LMA-G2, LMA-G3, Column LMA-A1 and Column LMA-A2 were fabricated (see Table S1). Columns LMA-G1 to G3 were fabricated to a length of 100 mm, 50 mm dedicated to photo-grafting and modification. Column LMA-A1 and LMA-A2 were fabricated with a total length of 200 mm, comprising