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Temperature control in large-internal-diameter scaffolded monolithic columns operated at ultra-high pressures

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ABSTRACT

Scaffolding makes it feasible to create organic-polymer monoliths in large confinements, such as wide-bore columns. By creating the scaffold from a metal good heat conductivity inside the column is obtained, which renders the relatively large columns (comparable with 4.6 mm i.d.) suitable for application under ultra-high-pressure LC conditions. It was anticipated that the metal scaffold would allow accurate control of the temperature within the columns, but the temperature profiles within the columns could not be characterized using the previously available small-internal-diameter scaffolded columns. In the current study the internal diameter of the scaffolded columns was increased up to square conduits of 4 × 4 mm. Prior to the formation of the stationary phase the heating efficiency in the empty scaffolded conduits was addressed. The performance of stationary phases created in the large scaffolds was investigated using the kinetic performance approach and the results were compared to those of the previous studies. Finally, scaffolded columns were tested under ultra-high-pressure LC conditions, where good temperature control is essential.

1. Introduction

Since their introduction in the 1990's [1], polymer-monolithic stationary phases have been progressively developed as alternative to packed-bed columns. Monolithic stationary phases were successfully applied for the separation of DNA fragments, peptides, proteins and antibodies [2–4]. The wide range of applications for large molecules originates from the polymeric structure. Organic monoliths feature interconnected polymer microglobules, typically in the sub-micron range, a distribution of macropores (flow-through pores), and a small number of mesopores (*i.e.* pores containing stagnant mobile phase), which implies a reduced stationary-phase contribution to mass-transfer (C_s-term) [5]. High-resolution biomolecule separations have mainly been demonstrated in small column formats, with internal diameters (i.d.) smaller than 1 mm [4]. The preparation of highly efficient, large-internal-diameter monolithic columns is still challenging. Shrinkage during polymerization typically amounts to 5 to 10 volume percent [6], which complicates the preparation of chromatographic materials in large, inflexible conduits. Furthermore, the application of solvent gradients causes the monolithic structures to swell and shrink repeatedly. This induces stress on the monolith, which may cause ruptures and possibly disconnection from the column walls, resulting in channelling effects and degrading separation performance [7].

To overcome these problems the possibility to create scaffolded monolithic materials was explored, as described in our previous study [8]. In earlier studies Desmet's group studied scaffolded pillar–array chips from silicon for creating stationary phases possessing porous-shell properties in 2D chips [9,10]. The 3D scaffolded structure for our study was created from titanium, a material that exhibits good heat-conducting properties, is inert under chromatographic conditions and allows fabrication of miniature designs. Furthermore, the polymer monolithic structure can be covalently linked to the scaffold surface after oxidation and chemical treatment with, for example, 3-(trimethoxy-silyl)propyl methacrylate (γ-MPS). The titanium-scaffold described in our previous study contained a square 1.3 × 1.3 mm conduit with a grid of 0.11 × 0.11 mm pillars placed 0.225 mm from each other in layers rotated by 90°, creating a well-defined, homogenous, three-dimensional structure. A macroporous poly(styrene-*co*-divinylbenzene) (S-*co*-DVB) monolith was created in the scaffold and covalently attached to the titanium surface. Because the distances spanned by the monolith are similar to those encountered in capillary formats the strength of the monolith was found to be greatly enhanced. This makes it plausible that titanium

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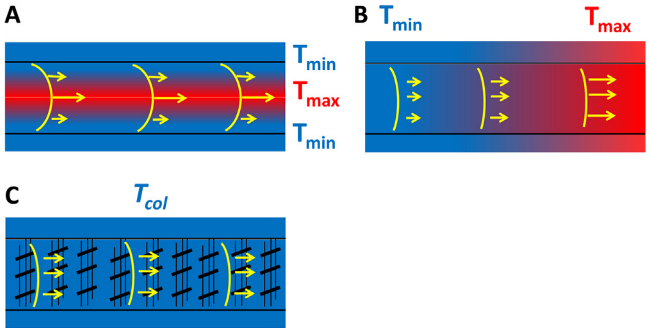

Fig. 1. The various temperature profiles observed when analytical columns are operated under UHPLC conditions. (A) Radial temperature gradients observed when operating a column under isothermal conditions; (B) Axial temperature gradient observed when operating the column under adiabatic conditions; (C) Ideal homogenous column temperature thought to be obtained by introducing a metal scaffold inside the column conduit.

scaffolding can be used to create large monolithic separation bodies, without disrupting the polymeric structure during polymerization or during solvent-gradient runs. The potential applicability of the narrow-bore titanium-scaffolded columns for the separation of intact proteins under ultra-high-pressure LC (UHPLC) conditions has been successfully demonstrated [8]. Nevertheless, the kinetic-performance limits obtained for the scaffolded columns were significantly lower than those of identical monoliths created in glass-lined tubing (0.8-mm i.d.).

Improved kinetic performance of analytical columns in terms of peak-capacity-per-unit-time is typically achieved by reducing the characteristic diameters, *i.e.* the particle size (d_p) for packed columns and the domain size for monolithic materials [11,12]. However, this is usually accompanied by a large increase in the operating pressure. When using a high pressure to force a flow through the interstitial voids of a chromatographic bed a great deal of heat is generated due to viscous friction [13]. Two limiting thermal conditions can be distinguished [13,14], defined as:(i) *isothermal conditions* where the column is perfectly thermostatted (*e.g.* using a water bath) and the generated heat is removed via the column wall:

$$\Delta T_{\text{in-outlet}} = \left(1 - \alpha \times \bar{T}\right) \times \frac{u_i \times \left(\Delta P/L\right) \times r^2}{8 \times \lambda} \tag{1}$$

and (ii) *adiabatic conditions* where the column is thermally insulated from surrounding air and no heat is removed or added via the column wall:

$$\Delta T_{\text{in-outlet}} = \left(1 - \alpha \times \bar{T}\right) \times \frac{\Delta P}{C_{p,v}} \tag{2}$$

where α is the thermal expansion coefficient of the mobile phase, u_i the superficial velocity, ΔP the column pressure drop, L the column length, r the column radius, λ the radial thermal conductivity and $C_{p,v}$ the volumetric heat capacity of the mobile phase [14,15]. When a column is operated under isothermal conditions the axial temperature gradient ($\Delta T = T_{\text{outlet}} - T_{\text{inlet}}$) will be small. However, the temperature in the centre of the column will be higher than the temperature near the column wall. An increased temperature results in a lower viscosity of the mobile phase and, consequently, a higher velocity in the centre of the column and an inhomogeneous flow profile (Fig. 1A). The result is a large additional contribution to the plate height [16]. Furthermore, Eq. 1 shows that viscous heating depends strongly on column internal diameter. In isothermal operation the plate height was found to vary with r^6 [14,17]. In contrast, when operating the column under adiabatic conditions (no heat transport; approached in a still-air oven; Fig. 1B) the axial temperature gradient is more significant, but the radial temperature gradient diminishes. This minimizes the additional band broadening but it will usually result in less retention and possibly slightly less resolution because retention factors decreases along the length of the column [14,16,17]. Radial temperature gradients are limiting maximum column dimensions that can be used [18,19], depending on the column-heating compartment used. Reduced system performance in UHPLC as a result of radial temperature gradients can already be observed in 2.1-mm i.d. columns [16,20]. Different solutions have been proposed to minimize detrimental effects of viscous heating on band broadening when operating large-internal-diameter columns under UHPLC conditions. Broeckhoven et al. coupled short columns and applied intermediate cooling to dissipate heat, reducing the axial temperature gradient by up to 50% [14]. Alternatively, stationary-phase particles with good heat-conductive properties have been proposed as an extra shell covering the classical silica materials [21].

In the present study the development of organic-monolithic stationary phases in large-internal-diameter scaffolds was explored. The metal scaffold is thought to circumvent the temperature gradients due to its good heat-conducting properties (Fig. 1C). The heat-transfer properties of the columns were studied by applying UHPLC conditions in isocratic mode. The effects of different porogen systems (THF/decanol and 3-methyl-1-butanol; *aka* isoamyl alcohol/decanol) and polymerization temperature on the resulting macroporous structure were investigated. Furthermore, the kinetic-performance limits of large-internal-diameter titanium-scaffolded monolithic columns were compared to those of small-internal-diameter scaffolded columns in gradient mode for the separation of intact proteins

2. Experimental

2.1. *Chemicals and materials*

Methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF, >99.8%), isopropanol (IPA) and acetone were purchased from Biosolve (Valkenswaard, The Netherlands). Sodium hydroxide (NaOH) was purchased from Merck (Darmstadt, Germany). Hydrochloric acid (HCl) (37%, (v/v)) was obtained from Acros (Geel, Belgium). Milli-Q water (18.2 MΩ cm^{-1}) was produced by a Sartorius Arium 611UV Ultrapure Water System (Goettingen, Germany). Styrene (S, >99.5%), divinylbenzene (DVB, 80%), 3-methyl-1-butanol (isoamyl alcohol, >98%), 2,2′-azobisiso-butyronitrile (AIBN, 98%), *n*-decanol (99%), 3-(trimethoxy-silyl)-propyl methacrylate (γ-MPS, 98%), aluminium oxide, toluene (99.9%), ribonuclease A from bovine pancreas, myoglobin from equine heart (>90%), lysozyme from chicken egg white (>90%) and carbonic anhydrase from bovine erythrocytes were purchased from Sigma Aldrich (Zwijndrecht, The Netherlands). All monomers were purified by passing them through a bed of activated basic alumina. Glass-lined tubing (0.3-mm and 0.8-mm internal diameter × 50 mm length) was purchased from Da Vinci Europe (Rotterdam, The Netherlands). The titanium-scaffold tubes (“small”: 1.3 × 1.3 × 50 mm; “large”: 4.0 × 4.0 × 80 mm) were developed for this project by FT Innovations (Boxmeer, The Netherlands). The titanium surface was chemically treated to create a titanium-oxide surface layer. ProSwift 1 × 50 mm was obtained from Thermo Fisher Scientific (Breda, The Netherlands). The reference columns (2.1 × 150 mm and 4.6 × 150 mm) packed with 1.7-μm particles were obtained from Waters (Milford, MA, USA).

2.2. In-situ *synthesis of monolithic stationary phases*

Macroporous poly(S-*co*-DVB) monolithic stationary phases were polymerized *in-situ* in the confines of the glass-lined tubing and titanium scaffolded columns, following an approach described