Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Short communication

Exploring bioimpendance instrumentation for the characterization of open tubular liquid chromatography columns

Øystein Skjærvø[a,b], Ole Kristian Brandtzaeg[b], Kristian Blindheim Lausund[b], Oliver Pabst[c], Ørjan G. Martinsen[c,d], Elsa Lundanes[b], Steven Ray Wilson[b,*]

[a] School of Pharmacy, University of Oslo, Post Box 1068, Blindern, NO-0316 Oslo, Norway
[b] Department of Chemistry, University of Oslo, Post Box 1033, Blindern, NO-0315 Oslo, Norway
[c] Department of Physics, University of Oslo, Post Box 1048, Blindern, NO-0316 Oslo, Norway
[d] Department of Clinical and Biomedical Engineering, Oslo University Hospital, 4950 Nydalen, 0424 Oslo, Norway

ARTICLE INFO

Article history:
Received 23 November 2017
Received in revised form
21 December 2017
Accepted 21 December 2017
Available online xxx

Keywords:
PLOT
Electrical resistance
Impedance
Porosity
Liquid chromatography
Pore blocking

ABSTRACT

Open tubular liquid chromatography columns with organic polymer layers can be powerful tools for high sensitivity measurements in *e.g.* proteomics. However, these narrow columns are challenging to characterize. A two-electrode system, often used for bioimpendance measurements, was used to study poly(styrene-co-divinylbenzene = PS-DVB) polymer layered open tubular (PLOT) liquid chromatography columns with 10 μm inner diameters. The system performed electrical resistance measurements (ERM) for assessing layer thickness and porosity. Layer determination results were comparable (but more precise) to that obtained with scanning electron microscopy (SEM). Porosity examinations with ERM casted doubt on the presence/availability of pores in the layers investigated.

1. Introduction

For the development and preparation of separation columns, reliable characterization methods are called for. We focus on open tubular liquid chromatography (OTLC) columns, which are capable of delivering very high chromatographic efficiency and sensitivity [1–3] for small molecules, peptides and intact proteins [1–5], making them powerful tools for *e.g.* metabolomics and proteomics of limited samples [4]. These columns are typically 10 μm or less in inner diameter (ID), often featuring a thin polymeric layer coating the inner walls, serving as a stationary phase. The narrow format allows for vastly reduced radial dilution, but is also required to minimize longitudinal dispersion that can seriously affect liquid-based separations in open tubular column format [6].

The traits of organic polymer layered OT columns (PLOT), *e.g.* layer thickness and porosity can be difficult to study. Scanning electron microscopy (SEM) [7] is commonly utilized, but thickness measurements should not always be taken at "face value", as SEM can give misleading images [8]: silica dissipate negative charge poorly, resulting in charge build-up that leads to image drift and distorted micrographs due to repulsion between the incoming electron beam and the charged sample ("charge effects"). In addition, when more electrons leave the sample at sharp edges compared to flat surfaces, some areas appear to be distorted or appear brighter than they should ("edge effects"). Furthermore, it is possible that the polymer layer stretches or deforms during SEM sample preparation, giving an inaccurate representation of the column sample.

Electrical resistance measurements (ERM) have been used to study biological components *e.g.* capillary blood vessels [9,10]. By measuring electrical properties between two electrodes, radius, porosity and other morphological features of the capillaries could be studied. The approach, which consists of fairly simple instrumentation, has also been used to *e.g.* characterize the blood brain barrier, membrane transport and muscle capillary endothelium [11–15]. ERM has (to the authorsí knowledge) not yet been used to study separation capillary columns. However, other electrical measurement approaches have been used to characterize polymers; Paull et al. [16] exploited capacitively coupled contactless conductivity detection (C4D) to monitor the polymer morphology of monolithic fused silica columns. The C4D method involved mix-

* Corresponding author.
E-mail address: stevenw@kjemi.uio.no (S.R. Wilson).

https://doi.org/10.1016/j.chroma.2017.12.060
0021-9673/

ing a salt solution in the polymerization mixture to study stationary phase growth by conductivity measurements between two electrodes. The C4D measurements provided promising results, but adding an ionic solution to the PLOT preparation could adversely affect the polymer growth (elaborated by the same authors in a more recent paper [17]). PLOT columns are also being investigated by other means, *e.g.* optical absorbance was used for *in situ* characterizations of PLOT polymer layers [17]. However, the method was reported to be problematic regarding lower ID capillaries.

Hence, alternatives/complementary approaches for characterizing very narrow PLOT columns would be highly convenient, and ERM is an unexplored option. We here report a platform for characterizing narrow PLOT capillaries with ERM, focusing on thickness layer and porosity.

2. Material and methods

2.1. Chemicals and materials

N,N-Dimethylformamide anhydrous (DMF), 3-(trimethoxysilyl)propyl methacrylate (γ-MAPS, 98%), divinylbenzene (DVB, 80% mixture of isomers), styrene (99%), sodium hydroxide (99%), 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and initiator 2,2 íazobis(2-methylpropinonitrile) (AIBN), [D-Ser4]-luteinizing hormone-releasing hormone (LHRH), octane (≥99%), decane (≥98%) and HPLC grade˜98% formic acid (FA) were all from Sigma Aldrich (Darmstadt, Germany). Ethanol absolute (EtOH, ≥99.98%) was from VWR (West Chester, PA, USA). HPLC grade ≥ 99.9% acetonitrile (ACN) was from VWR and type-1 water (resistivity 18.2 MΩ/cm at 25 °C) was from an ultrapure water purification system (Millipore Corporation, Billercia, MA, USA). Sodium chloride (99.5%) was from Merck (Darmstadt, Germany). Nitrogen gas 99.996% (4.6) was from AGA (Oslo, Norway). Fused silica capillaries (10, 15, 30, 50, 75 ± 2 μm ID, 363 ± 10 μm) outer diameter (the first 40 cm per capillary role was discarded to minimize production-related ID deviations) with polyimide coating were from Molex Polymicro Technologies™ (Phoenix, AZ, USA). Stainless steel (SS) 1/16“ unions (female) and Valcon ES 1.4 fused silica adapter 1/16” (for 0.4 mm outer diameter tubing) were from VICI (Houston, TX, USA). Polyether ether ketone (PEEK) tubings were from IDEX corp. (Lake Forest, IL, USA). 2-Propanol (anhydrous) was purchased from Kemtyl (Trollåsen, Norway).

2.2. Preparation of columns

PLOT columns with 10 μm ID were prepared according to previously described procedures [2,3] and an additional preparation procedure with a higher porogen to monomer ratio to reduce the thickness of the polymer layer. All solutions regarding column preparations were introduced by a pressurized system (4.6 grade nitrogen gas) made in-house at the Department of Chemistry, University of Oslo [18]. For each preparation procedure, four meters of fused silica capillary were filled with 1.0 M NaOH, sealed with rubber septum and laboratory film, and heated in an oven at 100 °C for two hours. The capillary was subsequently flushed with type-1 water followed by ACN to ensure that the capillary was free from residual water before drying with nitrogen. The silanization mixture was prepared from 0.3135 g γ-MAPS and 0.0050 g DPPH dissolved in 0.6608 g anhydrous DMF, and was ultrasonicated for 5 min prior to capillary introduction. After filling, the capillary was sealed and heated at 110 °C for six hours. Residual silanization mixture was removed with ACN before the capillary was dried by nitrogen. The silanized capillary was further cut into four specimens (∼1 m) before introduction of the polymerization mixture. The polymerization mixture was prepared according to three variations where 0.0050 g 2,2 íazobis(2-methylpropinonitrile), 0.1818 g styrene and 0.1828 g divinylbenzene were dissolved in A: 600 μL B: 942 μL and C: 1284 μL ethanol. The polymerization solutions were sonicated for five minutes prior to introduction. The capillaries were further sealed as above and heated at 74 °C for 16 h. After polymerization, the capillaries were flushed with ACN and dried with nitrogen. Polymerized capillaries were stored dry in a refrigerator prior to characterization.

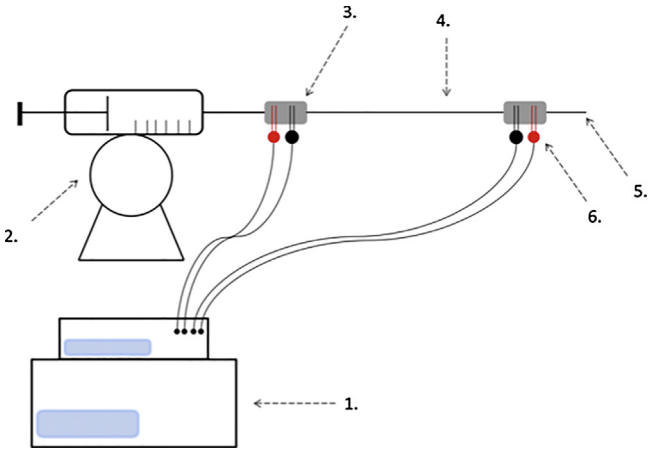

Fig. 1. The instrumental set-up used to measure the electrical resistance of an electrolyte solution passing through a capillary. The system consisted of: Solartron analytical SI1260 + 1294 impedance gain/phase analyzer (1), syringe pump with tip modified 250 μL syringe (2), 1/16” stainless steel unions (3), fused silica capillary/open tubular column (4), untreated capillary 10 μm ID (5) and crocodile clamps (6). The crocodile clamps were coupled to the gain/phase analyzer with standard electrical wiring.

2.3. Scanning electron microscopy

Polymerized fused silica specimens were cut in approximately 0.5 cm pieces and mounted with double sided carbon tape at an 80° angle using a vertical stage. SEM operations were done either with a Quanta 200 FEG (FEI, Hillsboro, OR, USA) in low vacuum mode with a 20 kV acceleration voltage or with a high resolution SU8230 CFEG instrument (Hitachi High-Technologies Corporation, Tokyo, Japan) at high vacuum with a 1 kV acceleration voltage or a landing voltage of 0.5 kV obtained using the instrument’s Deceleration mode. All micrographs were obtained using backscattered electron detectors. Layer thickness determinations with SEM measurements were based on the assessments of 6 persons for all specimens.

2.4. Electrical resistance measurement set-up

The overall instrument set-up used for the electrical resistance measurements is illustrated in Fig. 1. Fused silica capillaries of 10.0 cm length were examined. Electrolyte solution was introduced to the capillary by a kd Scientific (Holliston, MA, USA) syringe pump equipped with a 250 μL syringe (SGE analytical, Ringwood, Australia) featuring a blunt needle, which could be connected to a 1/16“ SS union using a 1/16” nut and a VICI 1/16” SS ferrule. The flushing procedure was carried out with 5 μL/min for 15 min for the electrolyte- and blocking solutions. The inlet of the capillary was connected to the union with a 1/16” nut and fused silica adapters (1/16”).

The outlet of the capillary was coupled to a 2 cm long fused silica capillary (un-treated, 10 μm ID), also using an SS union. Two electrodes (applied current and measured voltage for the outer and inner electrode pair, respectively) with crocodile clamps were coupled to each union. The electrodes were paired with a Solartron

Please cite this article in press as: Ø. Skjærvø, et al., Exploring bioimpendance instrumentation for the characterization of open tubular liquid chromatography columns, J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2017.12.060