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

Contents lists available at ScienceDirect

Journal of Chromatography A

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

A novel method for calibration of AF4 channels for hydrodynamic radius determination: The nanoemulsion method (featuring MALS)

Hans Bolinsson[a,*], Yi Lu[a], Stephen Hall[b], Lars Nilsson[a], Andreas Håkansson[c]

[a] Department of Food Technology, Engineering and Nutrition, Lund University, Lund, Sweden
[b] Division of Solid Mechanics, Lund University, Lund, Sweden
[c] Department of Food and Meal Science, Kristianstad University, Kristianstad, Sweden

ARTICLE INFO

Article history:
Received 12 September 2017
Received in revised form
23 November 2017
Accepted 10 December 2017
Available online xxx

Keywords:
Flow field-flow fractionation
AF4
Multi angle light scattering
Nanoemulsion
X-ray computed tomography
Channel height

ABSTRACT

This study suggests a novel method for determination of the channel height in asymmetrical flow field-flow fractionation (AF4), which can be used for calibration of the channel for hydrodynamic radius determinations. The novel method uses an oil-in-water nanoemulsion together with multi angle light scattering (MALS) and elution theory to determine channel height from an AF4 experiment. The method is validated using two orthogonal methods; first, by using standard particle elution experiments and, secondly, by imaging an assembled and carrier liquid filled channel by x-ray computed tomography (XCT). It is concluded that the channel height can be determined with approximately the same accuracy as with the traditional channel height determination technique. However, the nanoemulsion method can be used under more challenging conditions than standard particles, as the nanoemulsion remains stable in a wider pH range than the previously used standard particles. Moreover, the novel method is also more cost effective.

1. Introduction

Field flow fractionation (FFF) is a group of non-destructive separation techniques in which polydisperse samples can be fractionated for further investigations. A dispersed sample is injected in a channel, where separation is accomplished by a selective field directed perpendicular to the direction of sample and carrier liquid flow. For asymmetrical flow field flow fractionation (AF4) [1,2] the separating field is a cross flow, enabled by a permeable wall at the bottom of the channel. The permeable wall is defined by the pore size in the ultrafiltration membrane used. The membrane is supported by a frit, e.g. made from stainless steel. When applying a specific cross flow towards the permeable wall, the sample will relax by diffusion, at a translational diffusion coefficient dependent distances from the wall, and separate due to the laminar flow profile present in the channel during elution.

With the introduction of the asymmetrical trapezoidal separation channel in the 1990ś [3] followed a successful commercialization of AF4, which today is a versatile instrumentation with a wide application range [4–6]. The geometry of the trapezoidal channel allowed for improved efficiency, detection limits and resolution [3].

AF4 is often applied on colloidal systems and proteins as well as other macromolecules and particulate materials. AF4 is a non-destructive technique, allowing for the separation of ultra-high molar mass molecules and their aggregates. The effective separation range, in Brownian mode, is from a few nm to approximately 1000 nm. AF4 has the advantage of having no stationary phase, meaning low shear forces, small internal area and moderate pressure, as compared to other liquid chromatography techniques, e.g. size exclusion chromatography (SEC).

The hydrodynamic radius (r_{hyd}) of a sample can be determined directly from the AF4 retention time, provided knowledge of the channel height. Thus, with AF4 coupled to MALS the root mean square radius (r_{rms}) and r_{hyd} can then be determined simultaneously in one sample measurement. With these sample characteristics, r_{rms} and r_{hyd}, conformational information about the sample is revealed [7].

The maximum separation channel height is well known since it is defined by a spacer which also defines the trapezoidal geometry of the separation channel. However, the accumulation wall membrane is compressed by the spacer outside the channel perimeter, while inside, the membrane remains uncompressed. The carrier liquid can also induce some swelling of the membrane [8,9]. The amount of swelling and deformation depends on the carrier liquid

* Corresponding author at: Department of Food Technology, Engineering and Nutrition Lund University Box 124 SE-221 00 Lund.
E-mail address: hans.bolinsson@food.lth.se (H. Bolinsson).

https://doi.org/10.1016/j.chroma.2017.12.026
0021-9673/

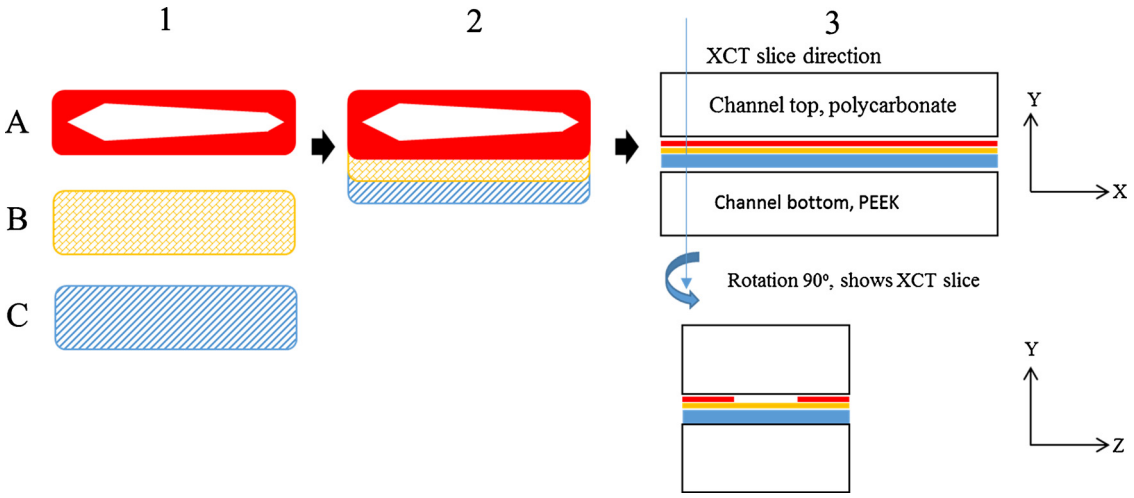

Fig. 1. Schematic illustration of the AF4 channel. Pane 1 shows the three layers A, B and C. A represents the spacer (250 μm), B represents the ultrafiltration membrane (permeable wall) and C represents the frit. Pane 2 shows the insertion order for channel assembly and pane 3 shows the assembled channel from the side. The XCT slice direction is indicated in the schematic. Rotating the slices 90 degrees shows the schematic view of the XCT slices. The elution channel volume is defined by the trapezoidal-shaped hole in the spacer, by the channel top and by the ultrafiltration membrane at the bottom.

characteristics and the type of membrane used [8,9]. Furthermore, channels are manually assembled and the tightening of the channel parts is also a source of variation. Consequently, it is irrelevant to measure the channel height before the channel is assembled or after it has been disassembled and a direct measurement of the channel height in the assembled channel is practically impossible. Fig. 1 illustrates the channel assembly.

All current methods of channel height determination require a calibration experiment with a particle of known hydrodynamic radius (or equivalently, translational diffusion coefficient). The retention time is measured for the sample and with the tabulated hydrodynamic radius the channel height can be calculated from retention theory [10–12]. Two types of calibration particles are typically used: small proteins (e.g. BSA and Ferritin) or larger polystyrene standard particles. Both alternatives have advantages and disadvantages.

Proteins are sensitive to pH and temperature, inducing changes to their hydrodynamic radius. Also, depending on the choice of carrier liquid, this can cause unwanted interactions between the protein and the membrane resulting in a size-independent shift of the retention time [13]. Polystyrene particles have been reported to require the addition of surfactant to the carrier liquid for successful elutions [14]. This can in many cases prohibit calibration of the channel height in the same carrier liquid as for the sample of interest. Both the proteins and polystyrene particles used are often considerably smaller than the molecules analyzed with AF4. Depending on the retention equation utilized, substantial systematic error in the determination of hydrodynamic radii may be introduced [10]. It should also be noted that this systematic error does not necessarily cancel out when using the obtained channel height in calculating hydrodynamic radius of the analyte, if the analyte and the calibration particle differ in size (see Fig. 2 in [10]).

In a recent review, Wahlund suggests using a range of different retention times to check the consistency in the determination of channel height [15]. However, this would require a larger number of calibration experiments or mixing several standards. In this study we suggest a new method for determining channel height using a polydisperse nanoemulsion aimed at incorporating this recommendation, with a single calibration experiment. In short, the method we propose provides estimates of channel height by fitting retention theory to the experimentally determined retention times using the entire emulsion drop-size distribution. Our proposed approach is expected to have several advantages. With a nanoemulsion, the calibration is performed with a distribution of sizes in one measurement giving consistency to the calibration. Emulsions can also be manufactured so that they have approximately the same size as the analytes under investigation, are stable over a long time period and can be stored in room temperature. In fact, even if the PSD of the emulsion would change over time, it would not interfere with the nano-emulsion method as any size changes would be detected by the light scattering detector used for the calibration. Moreover, emulsions formulated with suitable emulsifiers can make them less sensitive to changes in the carrier liquid properties.

The objective of this contribution is to present a new method for determination of channel height in AF4 experiments using a polydisperse emulsion, validate the method under different conditions, investigate stability to changes in the pH of the carrier liquid and discuss applicability to AF4. In order to provide high quality validation, direct imaging of the assembled channel using x-ray computed tomography (XCT) imaging has been employed. To the best of our knowledge, XCT has not previously been used for characterization of AF4 channels. An additional objective of this study is therefore to discuss applicability of this technique.

2. Experimental

2.1. Materials

Miglyol 812N was purchased from CREMER Oleo GmbH (Germany). Pluronic PE 6800 was obtained from BASF (Germany). Polystyrene particles (Catalog number 3060 A, certified mean diameter 59 ± 2 nm; Catalog number 3100 A, certified mean diameter 102 ± 3 nm; Catalog number R200, measured mean diameter 200 nm, CV < 5%) were purchased from Thermo Fisher Scientific (USA). Hereafter denoted PS60, PS100 and PS200 respectively. All analytical grade chemicals for buffer preparations were purchased from Sigma-Aldrich (USA).

2.2. Preparation of nanoemulsion

The nanoemulsion was prepared, adding 1% (v/v) Miglyol 812 N and 0.03% (w/v) Pluronic PE 6800 in H_2O MilliQ to a total volume of 100 ml. The emulsion mixture was then homogenized using an Ystral X-10/25 high shear mixer (Ystral GmbH, Germany) for 5 min

Please cite this article in press as: H. Bolinsson, et al., A novel method for calibration of AF4 channels for hydrodynamic radius determination: The nanoemulsion method (featuring MALS), J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2017.12.026