Factors affecting measurement of channel thickness in asymmetrical flow field-flow fractionation

Haiyang Dou [a,b,*], Euo Chang Jung [c], Seungho Lee [b,**]

[a] College of Basic Medical Sciences, Hebei University, Baoding 071-002, China
[b] Department of Chemistry, Hannam University, Daejeon 305-811, South Korea
[c] Nuclear Chemistry Research Center, Korea Atomic Energy Research Institute, Daejeon 305-353, South Korea

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ABSTRACT

Asymmetrical flow field-flow fractionation (AF4) has been considered to be a useful tool for simultaneous separation and characterization of polydisperse macromolecules or colloidal nanoparticles. AF4 analysis requires the knowledge of the channel thickness (w), which is usually measured by injecting a standard with known diffusion coefficient (D) or hydrodynamic diameter (d_h). An accurate w determination is a challenge due to its uncertainties arising from the membrane's compressibility, which may vary with experimental condition. In the present study, influence of factors including the size and type of the standard on the measurement of w was systematically investigated. The results revealed that steric effect and the particles–membrane interaction by van der Waals or electrostatic force may result in an error in w measurement.

1. Introduction

Field flow fractionation (FFF), a tool for the separation and characterization of particles and polymers, has attracted increasing interest in recent years owing to its broad dynamic range (approximately from 1 nm up to about 100 μm) and the utilization of "open channel" which requires no stationary phase or packing materials [1]. The sample degradation or loss is minimized in FFF, and there are fewer problems of sample adsorption than in size exclusion chromatography (SEC). Since its introduction, various subtechniques of FFF have emerged depending on the type of the external field employed [2–6]. Among them, flow FFF (FlFFF) has been considered to be the most versatile subtechnique, because the displacement of sample by the external field (cross-flow) is universal.

Asymmetrical FlFFF (AF4) has been extensively applied in various fields such as environmental study [7,8], food analysis [9,10], and life science [11,12]. In addition to separation, characterization of analytes by direct measurement of physicochemical parameters is one of the key features of FFF. Besides particle's diffusion coefficient (D) and hydrodynamic diameter (d_h), the conformation of polymers can also be evaluated based on the ratio of radius of gyration (r_g) to hydrodynamic radius (r_h), which can be obtained simultaneously by coupling AF4 with multiple detectors including the multiangle light scattering detector [13–15].

In AF4, theory is well established for size determination of analytes [6,16]. Size determination in the lift-hyperlayer mode requires a calibration using a series of size standards. In the normal mode, d_h can be directly calculated from measured retention time (t_r) and knowledge of experimental parameters such as the channel geometry and flow rates. Among the parameters, channel thickness (w) is one of critical parameters. According to AF4 theory, d_h is inversely proportional to w (see below in Eq. (8b)). Thus accurate measurement of w is required for determination of d_h. Also w affects the separation performance (e.g., resolution), as the separation efficiency (measured by the plate count N) increases with w when the cross-flow rate is held constant [17,18].

Usually w is smaller than the thickness of the channel spacer due to compressibility of porous ultrafiltration membrane used for the accumulation wall of the AF4 channel. The membrane is placed in the AF4 channel between the spacer and the frit that supports the membrane. The uncompressed portion of the membrane protrudes into the channel space resulting in the channel thickness w smaller than the spacer thickness as illustrated in Fig. 1.

There have been a few methods suggested for w determination in AF4, which were summarized in a recent publication [19]. Among them, the method proposed by Litzén [20] is commonly

* Corresponding author at: College of Basic Medical Sciences, Hebei University, Baoding 071-002, China. Tel.: +86 312 5075660; fax: +86 312 5937102.
** Corresponding author at: Department of Chemistry, Hannam University, Daejeon 305-811, South Korea.
E-mail addresses: douhaiyang-1984@163.com (H. Dou), slee@hannam.kr (S. Lee).
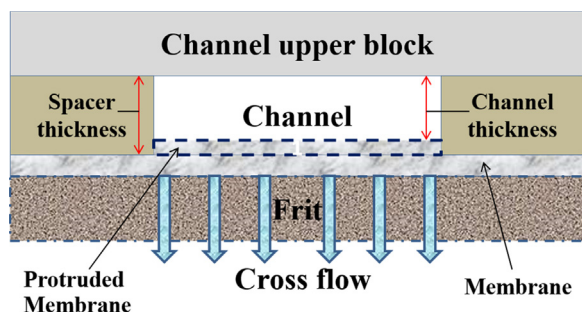
Fig. 1. Schematic cross-section of AF4 channel.

used for w determination, which was established with some underlying assumptions such as strong retention and no steric effect [21]. Wahlund [19] had shown the reliability of Litzén's method under certain experimental conditions. In practice, however, w measurement is not always straightforward since non-idealities may exist. Sometimes, experimental conditions do not allow the assumptions leading to the 'simplified' retention equation to be fulfilled, and thus the accuracy in sample characterization is impaired. Giddings [22] and Martin [23] have outlined systematically the factors which may give rise to departure from classical retention theory of FFF. Unfortunately, no systematic work has been reported to investigate the factors influencing the accuracy of w determination.

Often w is determined using a standard sample with known D or d_h without much attention to whether experimental conditions satisfy the underlying assumptions. Sometimes w measured at an experimental condition is used to determine d_h from data obtained at different conditions without providing detailed information on how w was determined. Without knowledge on the influence of experimental conditions (such as the carrier liquid composition and flow rates), it may introduce an ambiguity into AF4 data treatment such as determination of d_h [24,25].

It is thus important to understand the factors that limit accurate determination of w. In this work, the influence of factors on w determination was investigated systematically. The objective of this work is to study the influence of a variety of parameters affecting the w determination in AF4.

2. Theory

In AF4, the field force F exerted on sample components is expressed by [16]:

$$F = \frac{V_c w k T}{V^0 D},\tag{1}$$

where V_c is the cross-flow rate, w is the channel thickness, k is the Boltzmann constant, T is the absolute temperature, and V^0 is the void volume. Sample components are pushed toward the accumulation wall (membrane) by the field force. At the same time, the components diffuse away from the accumulation wall by Brownian motion, and eventually form an equilibrium layer between the two opposing transport processes. The mean layer thickness (l) equals to the distance from the accumulation wall to the center of gravity of the equilibrium layer as highly compressed analyte layer is close to the accumulation wall.

In FFF experiments, retention ratio (R) is obtained by [6]:

$$R = \frac{t^0}{t_r},\tag{2}$$

where t^0 and t_r are 'void time' and 'retention time', respectively. In FFF theory, R is expressed by [6]:

$$R = \frac{6}{w}\frac{\int_0^w e^{(-x/l)B(x)}x\,dx - \frac{1}{w}\int_0^w e^{(-x/l)B(x)}x^2\,dx}{\int_0^w e^{(-x/l)B(x)}\,dx},\tag{3}$$

where

$$B(x) = 1 - \frac{x^2}{w^2} + \frac{x^3}{2w^3}\tag{4}$$

Due to the complexity of Eq. (3), R cannot be obtained in a closed form and therefore has to be evaluated numerically. In cases of high retention, $B(x)$ could be assumed to be unity. Assuming $B(x) = 1$ and there is no steric effect, Eq. (3) is simplified to [17]:

$$R = 6\lambda\left[\coth\left(\frac{1}{2\lambda}\right) - 2\lambda\right],\tag{5}$$

where λ is the dimensionless retention parameter, which is expressed in AF4 by [26]:

$$\lambda = \frac{kTV^0}{3\pi\eta V_c w^2 d_h}\tag{6}$$

For highly retained components in AF4, meaning $l \ll w$ or $\lambda \to 0$, Eq. (5) can be approximated to yield a so-called 'simplified' retention equation:

$$R = 6\lambda\tag{7}$$

Retention equations can be used to determine λ from measured t_r, then to determine d_h. Using Eqs. (2), (6) and (7), d_h can be determined directly from measured t_r by:

$$d_h = \frac{2kTV^0}{\pi\eta V_c w^2 t^0}t_r\tag{8a}$$

Substituting $V^0 = Aw$ (A is the area of the accumulation wall) into Eq. (8a) yields:

$$d_h = \frac{2kTA}{\pi\eta V_c w t^0}t_r\tag{8b}$$

Eq. (8) is valid to within 1% when $R \le 0.029$, within 5% when $R \le 0.17$, and within 10% when $R \le 0.44$ [27]. Eq. (8) shows a linear relationship between d_h and t_r with the proportionality constant depending on the experimental conditions such as the channel geometry, flow rate, and the channel thickness w. In practice, w can be determined from Eq. (8) using a standard sample with known size (d_h) such as commercial polystyrene (PS) latex beads [28,29].

An alternative is to inject a standard sample with known D [20], where w is determined by:

$$w = \sqrt{\frac{6Dt_r}{\ln\left\{1 + \frac{V_c}{V_{out}}\left[1 - \frac{b_0 z' - \left(\frac{b_0 - b_L}{2L}\right)z'^2 - y}{A}\right]\right\}}},\tag{9}$$

where the V_{out} is the channel outlet flow rate, b_0 and b_L are the breadths of the trapezoid, z' is the distance from tip of the channel inlet to the focusing point, L is the channel length, y is the area lost from the trapezoid by the tapered inlet and outlet ends. In both methods, a simplified retention equation (Eq. (7)) is employed, where it was assumed that the sample components are mass points and there is no inter-component interaction and component–membrane interaction [22]. Thus a finite component sizes and presence of component–membrane interaction may induce an error in w determination, and eventually in size determination.