Precise batch evaluation of Asymmetrical Flow Field-Flow Fractionation data via automatized void peak determination – Supplementary information

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Abstract

Asymmetrical field-flow fractionation is a versatile chromatographic fractionation method. In combination it is used for size-based separation of colloids, biomolecules and polymers. Although used often as pure separation method, a well-elaborated theory is available that allows precise quantification of the analysis results. A conversion from the time domain to the domain of hydrodynamic radius yields size distribution directly from the fractogram. However, up to now, this is an error-prone procedure requiring some effort as the fractrograms have to be pre-processed manually to gain all information required for the conversion. In this work, we present a software-based evaluation workflow which circumvents these pitfalls allowing to calculate reliable distributions. Providing a small graphical user interface minimizes the manual effort of evaluation which turns out to be useful especially for method development, extensive parameter studies and multi-detection methods.

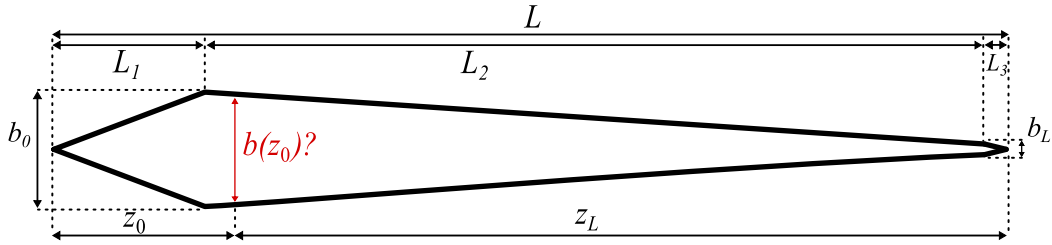## Determination of geometrical channel Volume V^{geo}

Fig. S.1: Channel dimensions. The width $b(z_L)$ depends on the focus position and is not accessible via bare channel data.

AF4 channel have a trapezoidal shape with measures indicated in fig. S.1. For all further considerations, the channel plane is split into three sections (1,2,3) with their correspoding lengths L_1, L_2, L_3. To simplify the further calculations, they are subsumed as in the following:

$$L = L_1 + L_2 + L_3 = L_{12} + L_3 \tag{S.1}$$

As the sample is focussed at a certain channel position on the beginning, this has to be considered. The relative focus position $z_\%$ is related to the other focus-related magnitudes by
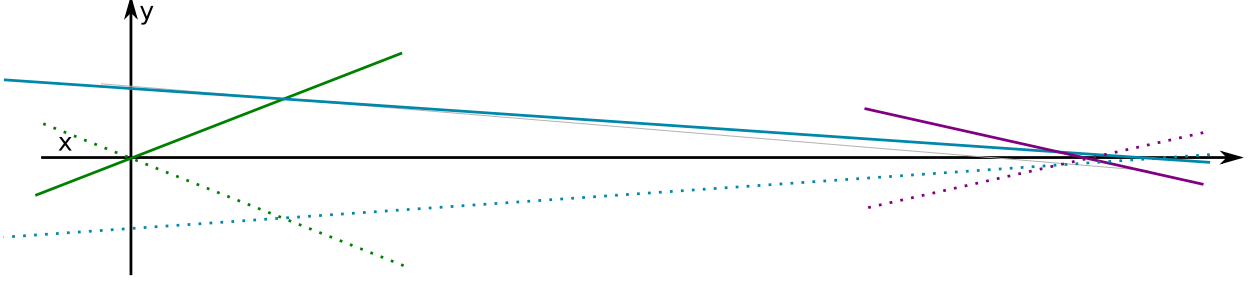
$$z_0 = z_\% L = L - z_L \tag{S.2}$$

Fig. S.2: Channel dimensions as a set of 3 pairs of straight lines

The channel height difference b_Δ on the section 2 is

$$b_\Delta = b_L - b_0 \tag{S.3}$$

Volume calculation may be conducted for the trapezoidal by simple decomposition of the channel plane into elementary geometrical objects. However, a concise analytical approach is more appropriate as the result can be displayed as a function of zP. In addition the corresponding $b(zP)$ is not known initially. Similar derivations have already been conducted with the approximation of dividing the shape into two sections.[1–4] The approach may be useful for further hydrodynamic considerations as for example, the elution flow $V_e(x)$ in AF4 is a position-dependent size. For the trapezoidal plane shape, the channel is described by the enclosure of three pairs of straight line S.2. All expressions here are not optimized for mathematical elegance, but rather for being translated into an understandable and well-maintainable calculation routine. This is achieved by heavy subsitution of the known variables are early subsumed to simplify the later expressions. Due to the reason of symmetry, only three borders have to described exactly:

$$\frac{1}{2}b(x) = E(x) \begin{cases} e_1(x) = m_1 x = \frac{b_0}{2L_1} \cdot x & \forall & x \leqq L_1 \\[2mm] e_2(x) = m_2 x + t_2 = -\frac{b_\Delta}{2L_2} \cdot x + \frac{1}{2}\left(b_0 + \frac{L_1}{L_2}b_\Delta\right) & \forall & L_1 < x \leqq L_{12} \quad \text{(S.4)} \\[2mm] e_3(x) = m_3 x + t_3 = -\frac{b_L}{2L_3} \cdot x + \frac{Lb_L}{2L_3} & \forall & L_{12} < x \leqq L \end{cases}$$

As all dimensions here are known, the slopes and offsets of the lines can be calculated directly and don't have to be resubsituted after the following substitutions. The calcuation of geometrical Volume of the trapezoidal channel has to be adapted according to whether the focus position z_L is located left or right to the position of maximal channel extent (i.e. if $z_L < L_1$ or $z_L \geqq L_1$).
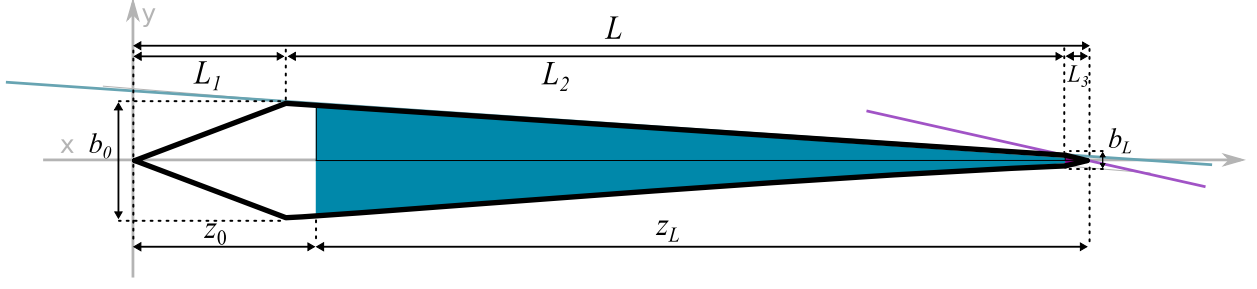
Distal focussing with $z_0 \geqq L_1$

Fig. S.3: Case 2

In this case, the effective separation volume is the colored area in the x, y-plane of Fig. S.3: The channel volume V^{geo} is described by

$$V^{\mathrm{geo}} = \left(\boxed{A_2} + \boxed{A_3} \right) \cdot w$$

$$= 2 \cdot \left(\boxed{\int\limits_{z_0}^{L_{12}} e_2(x) \, \mathrm{d}x} + \boxed{\int\limits_{L-L_3}^{L} e_3(x) \, \mathrm{d}x} \right) \cdot w$$

$$= \left(\boxed{(L_{12} - z_0)(m_2(L_{12} + z_0) + t_2)} + \boxed{\tfrac{1}{2} \cdot L_3 \cdot b_L} \right) \cdot w \qquad \text{(S.5)}$$

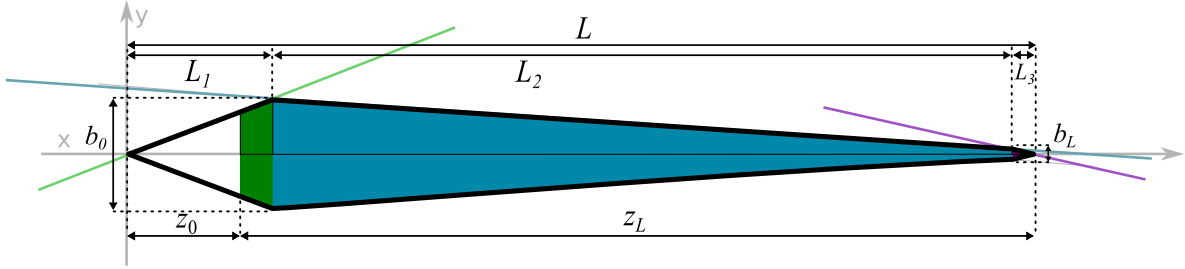**Proximal focussing with $z_0 < L_1$**

Fig. S.4: Case 2

Outgoing from the previous result, the full area section of section 2 has to be considered, i.e. first z_0 is replaced by L_1, then the area part of section 1 is added:

$$V^{\mathrm{geo}} = \left(\boxed{A_1} + \boxed{A_2} + \boxed{A_3} \right) \cdot w$$

$$= 2 \cdot \left(\boxed{\int\limits_{z_0}^{L_1} e_1(x) \, \mathrm{d}x} + \boxed{\int\limits_{L_1}^{L_{12}} e_2(x) \, \mathrm{d}x} + \boxed{\int\limits_{L-L_3}^{L} e_3(x) \, \mathrm{d}x} \right) \cdot w$$

$$= \left(\boxed{m_1 \cdot (L_1^2 - z_0^2)} + \boxed{L_2(m_2(L_{12} + L_1) + t_2)} + \boxed{\tfrac{1}{2} \cdot L_3 \cdot b_L} \right) \cdot w \qquad \text{(S.6)}$$

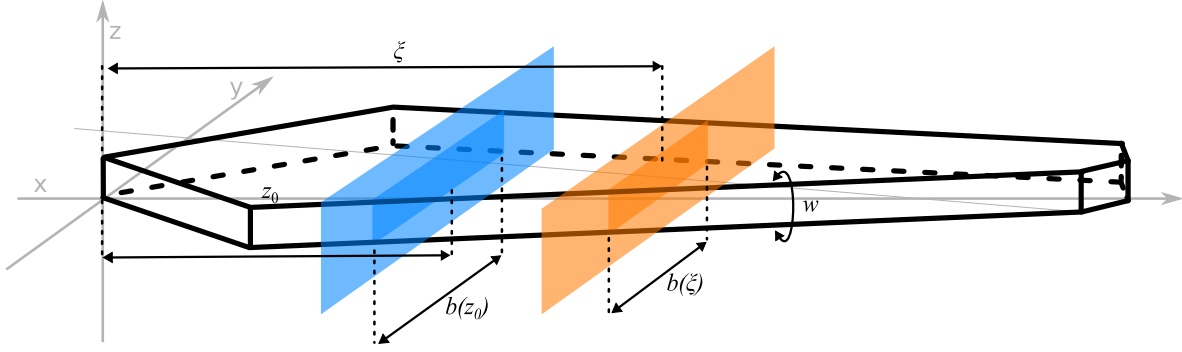## Determination of "hydrodynamic" Volume V^{hyd}

Fig. S.5: Case 2

Here, the derivation is analogously conducted as described in literature[1, 5], but using the straight equations S.4 for description of the channel above. t_{void} is the void time of an unretained species which can be obtained by integration over the channel positions ξ

$$t_{\mathrm{void}} = \int_0^{t_{\mathrm{void}}} \mathrm{d}t = \int_{z_0}^L \frac{1}{v_{\mathrm{m}}(\xi)} \, \mathrm{d}\xi \tag{S.7}$$

$v_{\mathrm{m}}(\xi)$ is the migration velocity of the eluent at a channel position $v_{\mathrm{m}}(\xi)$. It dependends on the flow velocity $\dot{V}(\xi)$ at the position and the y, z-crossesctional area $B(\xi)$ at (Fig S.5)

$$v_{\mathrm{m}}(\xi) = \frac{\dot{V}(\xi)}{B(\xi)} = \frac{\dot{V}(\xi)}{b(\xi) \cdot w} \tag{S.8}$$

The term $b(\xi)$ is described with the aid of eq. S.4 and will require a case-by-case approach. The change of the flow velocity $\dot{V}(\xi)$ is exactly the total loss in the applied crossflow. It has its maximum at the inlet position with

$$\dot{V}(0) = \dot{V}_{\mathrm{in}} = \dot{V}_{\mathrm{e}} + \dot{V}_{\mathrm{c}} \tag{S.9}$$

and its minimum with

$$\dot{V}(L) = \dot{V}_{\mathrm{e}} \tag{S.10}$$

As this is distributed uniformly over the membrane surface, the decay is proportional to the area the eluent has already passed. This leads to the expression

$$\dot{V}(\xi) = \dot{V}_{\mathrm{in}} - V_c \cdot \frac{A(\xi)}{A_L} = \dot{V}_{\mathrm{in}} - V_c \cdot \frac{\int_0^\xi b(x)\,\mathrm{d}x}{\int_0^L b(x)\,\mathrm{d}x} = \dot{V}_{\mathrm{in}} - V_c \cdot \frac{2 \cdot \int_0^\xi E(x)\,\mathrm{d}x}{A_L} \tag{S.11}$$

The total area A_L can be easily derived by letting of eq. S.6 with letting $z_0 = 0$:

$$A_L = \left(\boxed{\tfrac{1}{2} b_0 L_1} + \boxed{L_2 \left(m_2 \left(L_{12} + L_1 \right) + t_2 \right)} + \boxed{\tfrac{1}{2} \cdot L_3 \cdot b_L} \right) \cdot w \tag{S.12}$$

To evaluate $A(\xi)$ correctly, the integrals have to be splitted according to the conditions in eq. S.4. This is required which corresponds to the cases needed for $b(\xi)$. Merging eq.and S.11 gives the

expression

$$v_{\mathrm{m}}(\xi) = \frac{\dot{V}_{\mathrm{in}} - V_c \cdot \dfrac{2 \cdot \displaystyle\int_0^\xi E(x)\,\mathrm{d}x}{A_L}}{2 \cdot E(\xi) \cdot w} \tag{S.13}$$

Inserting Eq.S.7 gives:

$$t_{\mathrm{void}} = \int_{z_0}^L \left(\frac{2 \cdot E(\xi) \cdot w}{\dot{V}_{\mathrm{in}} - V_c \cdot \dfrac{2 \cdot \displaystyle\int_0^\xi E(x)\,\mathrm{d}x}{A_L}} \right) \mathrm{d}\xi \tag{S.14}$$

UML class diagram

Perhaps, but not mandatory

User interface

2 images

Detailed description of applied algorithms

Calibration of channel height by $V^{\mathbf{hyd}}$

Inputs:

- void peak t_{void}

- elution flow $\dot{V}_{\mathrm{e}}$
- elution time t_{e}
- cross flow $\dot{V}_{\mathrm{c}}$

- diffusion coefficient D
- focus point $z_\%$;

Outputs:

- channel width w

- channel volume V^{hyd}

Internal variables:

- measured retention

R_{meas}
- variation Δ
- λ

- $w_{\mathrm{min}} \leftarrow 10^{-4}$
- $w_{\mathrm{max}} \leftarrow 10$

Calculations:

1 Calculate volume:
NEW FORMULA

2 Calculate R_{meas}:

$$R_{\mathrm{meas}} \leftarrow \frac{t_{\mathrm{void}}}{t_{\mathrm{e}}}$$

3 Initialize w and Δ:

$$w \leftarrow \frac{w_{\mathrm{max}} + w_{\mathrm{min}}}{2}$$

$$\Delta \leftarrow \frac{w_{\mathrm{max}} - w_{\mathrm{min}}}{4}$$

4 Find w such that $|R_{\text{meas}} - R_{\text{calc}}| \overset{!}{=} \min$ by bisection:

for $i \leftarrow 0$ to 50 **do**
    $\lambda \leftarrow \frac{D \cdot V^0}{V_C \cdot w^2}$
    $R_{\text{calc}} \leftarrow 6\lambda \left(\frac{1}{\tanh(1/2\lambda)} - 2\lambda \right)$ # $^1/_{\tanh(x)} = \coth(x)$
    if $R_{\text{calc}} > R_{\text{meas}}$ **then**
        $w \leftarrow w + \Delta$
    else
        $w \leftarrow w - \Delta$
    end if
    $\Delta \leftarrow \Delta/2$
end for

Calibration of channel height by V^{geo}

xxxxxxxxxxxxxxxxxxxxxxxxxxxx

Axis conversion of $t \longrightarrow D \longrightarrow R_S$

Inputs:

- data point index j
- time t_j
- volume $V^0 (V^{\text{geo}} \text{or} V^{\text{hyd}})$
- focus point $z_\%$;
- cross flow $\dot{V}_{\text{c}}$
- channel width w
- temperature T
- viscosity η

Outputs:

- D_j
- $R_{\text{S}}j$

Internal Variables:

- void Peak t_{void}
- measured retention R_{meas}
- variation Δ
- λ

Calculations:

1 Calculate t_{void}:
NEW FORMULA!

2 Find such D_j that $|R_{\text{meas}} - R_{\text{calc}}| \overset{!}{=} \min$ by bisection:

for all $t_j > t_{\text{void}}$ **do**
    $R_{meas} \leftarrow \frac{t_{\text{void}}}{t_e - t_{\text{void}}}$
    for $k \leftarrow 0$ to 50 **do**
        $\lambda \leftarrow \frac{D \cdot V^0}{V_C \cdot \omega^2}$
        $R_{calc} \leftarrow 6\lambda \left(\frac{1}{\tanh(1/2\lambda)} - 2\lambda \right)$ # $^1/_{tanh(x)} = \coth(x)$
        if $R_{calc} > R_{Meas}$ **then**
            $D_j \leftarrow D_j - \Delta$
        else
            $D_j \leftarrow D_j + \Delta$
        end if
        $\Delta \leftarrow \Delta/2$
    end for
end for

3   Calculate R_{Sj}:

for all D_j **do**

$\qquad R_{Sj} \leftarrow \frac{k_B \cdot T}{6\pi \cdot \eta D_j}$

end for

Complete data sets

Detailed measurement method

Sequence order

3   Calculate R_{Sj}:

for all D_j **do**

$\qquad R_{Sj} \leftarrow \frac{k_B \cdot T}{6\pi \cdot \eta D_j}$

end for

References

[1] K.-G. Wahlund, *Journal of chromatography. A* **2013**, *1287*, 97–112.

[2] E. Magnusson, A. Håkansson, J. Janiak, B. Bergenståhl, L. Nilsson, *Journal of chromatography. A* **2012**, *1253*, 127–133.

[3] H. Bolinsson, Y. Lu, S. Hall, L. Nilsson, A. Hakansson, *Journal of chromatography. A* **2018**, *1533*, 155–163.

[4] A. Håkansson, E. Magnusson, B. Bergenståhl, L. Nilsson, *Journal of chromatography. A* **2012**, *1253*, 120–126.

[5] K. G. Wahlund, J. C. Giddings, *Anal Chem* **1987**, *59*, 1332–1339.