SOFTWARE METAPAPER

Flow Rates in Liquid Chromatography, Gas Chromatography and Supercritical Fluid Chromatography: A Tool for Optimization

Joris Meurs

Department of BioAnalytical Chemistry, VU University Amsterdam, NL
j.meurs@student.vu.nl

This paper aimed to develop a standalone application for optimizing flow rates in liquid chromatography (LC), gas chromatography (GC) and supercritical fluid chromatography (SFC). To do so, Van Deemter's equation, Knox' equation and Golay's equation were implemented in a MATLAB script and subsequently a graphical user interface (GUI) was created. The application will show the optimal flow rate or linear velocity and the corresponding plate height for the set input parameters. Furthermore, a plot will be shown in which the plate height is plotted against the linear flow velocity. Hence, this application will give optimized flow rates for any set conditions with minimal effort.

(1) Overview

Introduction

Liquid chromatography (LC), gas chromatography (GC) and supercritical fluid chromatography (SFC) cover a wide area for quantitative analysis of organic compounds in analytical chemistry [1]. For quantification of a compound the system needs a certain resolution and a suitable detector. In an ideal case, peaks are narrow and follow a Gaussian distribution. However, several factors are contributing to peak broadening like detector, column, mobile phase, injection, column temperature and flow rate [2]. To get an optimal performance of the LC, GC or SFC system the theoretical plate height (HETP) should be minimized. Van Deemter [3], Knox [4] and Giddings [5] developed theories for calculating the contributions to peak broadening in a packed column. Mostly, Van Deemters equation and Knoxs equation are used for calculating the HETP based on the flow velocity [6]. For capillary columns, Golay [7] developed an equation for HETP calculation.

Since these calculations are time-consuming and susceptible for errors, this paper aimed to develop an application in which this calculation can be performed quickly. To do so, Van Deemter's equation, Knox' equation and Golay's equation were chosen for calculating the HETP for packed and capillary columns respectively. These equation were implemented in a MATLAB algorithm and thereafter a Graphical User Interface (GUI) was developed.

Theory

Packed columns

Van Deemter's equation
According to Van Deemter, the HETP (H) can be calculated as follows [8]:

$$H = 2\lambda d_p + \frac{2\gamma D_m}{u} + \left(\frac{f(k)d_f^2}{D_s} + \frac{f(k)d_p^2}{D_m} \right) u \quad (1)$$

Which is often reduced to:

$$H = A d_p + \frac{B D_m}{u} + \frac{C u d_p^2}{D_m} \quad (2)$$

Equation 1 describes band broadening by eddy diffusion (A), longitudinal diffusion (B), mass transfer (C) at different flow velocities in a packed column. Eddy diffusion is dependent on the quality of the packing of the column [8]. A well-packed column has an A-term value of about 1 [9].

Longitudinal diffusion occurs when a concentration gradient exists. Analyte molecules will migrate from a high concentration area to a low concentration area according to Ficks law. This process is dependent on the diffusion coefficient of the mobile phase (D_M) and a constant, which is dependent on the quality of the column [8]. A common value for the B-term is 2 [9].

Mass transfer is a complex process, which is described by a function f, the derivative of the function f, the particle size (d_p), the diffusion coefficient of the mobile phase (D_M), the diffusion coefficient of the stationary phase (D_s) and the thickness of the liquid layer on the stationary phase (d_l) [8]. The function of f is dependent on the retention factor (k). In general, the values for C is 0.05 [9].

To obtain the minimum value of the HETP, Equation 2 should be differentiated with respect to u and solved for dH/du = 0. Differentiation of Equation 2 resulted in:

$$\frac{\partial H}{\partial u} = \frac{Cd_p^2}{D_m} - \frac{BD_m}{u^2} \qquad (3)$$

The result of Equation 3 should then be used in Equation 2. To do so, has to be Equation 2 rewritten to a function in which the linear velocity is expressed as a function of the HETP:

$$u = \frac{Z + D_m H - AD_m d_p^2}{2Cd_p^2} - \frac{Z - D_m H + AD_m d_p^2}{2Cd_p^2} \qquad (4)$$

where,

$$Z = D_m \sqrt{A^2 d_p^2 - 2Ad_p H - 4BCd_p^2 + H^2} \qquad (5)$$

However, more often one uses reduced parameters for calculating the optimal flow rate since these parameters can be easily transferred between system. The reduced Van Deemter equation is defined as follows [9]:

$$h = A + \frac{B}{v} + Cv \qquad (6)$$

The reduced plate height (h) is defined by respectively the A-term, B-term, C-term and the reduced velocity. As can be derived from this equation, h is independent of the particle size and diffusion coefficient. The A-term, B-term and C-term can be calculated according to the following equations [10]:

$$A = 2\lambda d_p \qquad (7)$$

$$B = 2\gamma D_G \qquad (8)$$

$$C = \frac{8}{\pi^2} \frac{k}{(1+k)} \frac{d_L^2}{D_L} \qquad (9)$$

Where, λ is the correction factor for the irregularity of the column packing, d_p is the particle size, γ is the labyrinth factor (value between 0 and 1), D_G is the diffusion coefficient of the analyte in the gas phase, k is the retention factor, d_L is the thickness of the stationary phase layer and D_L is the diffusion coefficient of the analyte in the stationary phase [10].

Knox' equation

Another well-used equation for calculating the contribution of flow rates to band broadening is the Knox equation [4]. This equation only consists of reduced parameters (Equation 2.7) [11]:

$$h = Av^{1/3} + \frac{B}{v} + Cv \qquad (10)$$

In contrast to the Van Deemter equation, the A-term is dependent on the reduced velocity (v) according to Knox [11]. Common for the A-term, B-term and C-term are 1, 2 and 0.05 respectively according to Knox [9, 11].

Capillary columns

The separation efficiency of a capillary column can be calculated by using Golay's equation [12]:

$$H = \frac{2D_m}{u} + f(k)\frac{d_c^2 u}{D_m} + g(k)\frac{d_f^2}{D_f} \qquad (11)$$

In which D_m is the diffusion coefficient of the mobile phase, u is the linear velocity, D_f is the diffusion coefficient of the film, d_c is the diameter of the column and d_f is the film thickness. Further, f and g are functions of the retention factor (k). These functions are described below [12]:

$$f(k) = \frac{1 + 6k + 11k^2}{96(1-k)^2} \qquad (12)$$

$$g(k) = \frac{2k}{3(1-k)^2} \qquad (13)$$

To minimize the H, Equation 11 should differentiated with respect to u and the derived equation should be solved for $u = 0$. The final result of differentiating Equation 11 with respect to u is as follows:

$$\frac{\partial H}{\partial u} = \frac{d_c^2 f(k)}{D_m} - \frac{2D_m}{u^2} + \frac{d_f^2 g(k)}{D_f} \qquad (14)$$

In **Table 1** the units per variable and common values for the variables are shown respectively.

Diffusion coefficients of the mobile phases used in LC, GC and SFC have a magnitude of $10^{-9}\ m^2 \cdot s^{-1}$, $10^{-6}\ m^2 \cdot s^{-1}$ and $10^{-8}\ m^2 \cdot s^{-1}$ respectively [12].

Implementation and architecture

First, a blank graphical user interface (GUI) was generated (MATLAB R2016a) in which a pop-up menu was added for accessing all calculation sections. After, the equations described in Section 1 were implemented into corresponding section of the script. For example, Equation 1 is implemented in the section 'Liquid Chromatography' (packed column). To ensure only relevant data can be given as input, limitations have been set per calculation section. The limitations for Equation 1 are shown below. For calculation, first linear spaced data (`linspace`) is generated representing the linear flow velocity. Subsequently, the theoretical plate height is calculated. Thereafter, the minimum plate height is determined by using the `min` function. In order to find the corresponding linear flow velocity, the data of the linear flow velocity and the corresponding theoretical plate heights are placed into a matrix. Using `find`, the row of the minimal plate

Type of column	Variable	Unit
Packed column		
	A	Dimensionless
	B	Dimensionless
	C	Dimensionless
	D_m	$m^2 \cdot s^{-1}$
	u	$m \cdot s^{-1}$
	d_p	μm
	k	Dimensionless
Capillary column		
	d_c	mm
	D_m	$m^2 \cdot s^{-1}$
	D_f	$m^2 \cdot s^{-1}$
	u	$m \cdot s^{-1}$
	d_p	μm
	d_f	μm
	k	Dimensionless

Table 1: Overview of units per variable.

height is determined. After, the corresponding value for the linear velocity can be determined. This value is converted to the optimal flow rate in m³/min, which is subsequently converted to mL/min. After calculation, the optimal flow rate, optimal linear flow velocity and minimal plate height will be displayed. Additionally, a plot will be generated in which the theoretical plate height is plotted against the linear flow velocity.

Quality control

In order to check the performance of the application, guidelines for flow rate optimization in liquid chromatography were used from [13]. The optimal flow rates per internal diameter of a packed column containing 5μm particles is shown in **Table 2**.

These guidelines were used to perform test calculations. For these calculations, A, B, C, the diffusion coefficient of the mobile phase and the volume fraction were set at 2, 1, 0.05, $2.369 \cdot 10^{-9}$ m²/s and 0.7 respectively. The results of the calculations can be found in **Table 3**. The example data can be accessed via the code repository.

From **Table 3** can derived that the application shows results which are in accordance with the guidelines

Internal diameter (mm)	Optimal flow rate (mL/min)
2	0.15–0.50
3	0.60
4 – 4.6	0.80–2.0
8	2.0–4.0

Table 2: Optimal flow rates for packed columns with 5μm particles at different internal diameters according to [13].

Internal diameter (mm)	Optimal flow rate (mL/min)	Calculated flow rate (mL/min)
2	0.15 – 0.50	0.28
3	0.60	0.63
4	0.80–2.0	1.11

Table 3: Results of test calculations compared to guidelines of [13].
Calculated values are rounded to two decimals.

from [13]. In two cases, the calculated results deviate, but do not show unreasonable results.

As is mentioned in the Implementation and Architecture section, limitations are set in order to enhance the quality of the calculations. Furthermore, by clicking on the push buttons, information can be obtained on common values. A brief tutorial (`How-To-Use.pdf`) of the application can be found in the code repository.

(2) Availability

Operating system
The GUI was originally designed in MATLAB R2016a (Windows) and later also tested in MATLAB R2010a (Windows) without any problems. Furthermore, the `LC_GC_SFC.m` file was tested on a Linux system with MATLAB R2014b and MATLAB R2016a without any problems. Later, the GUI was compiled to an executable file (.exe) for Windows only.

Programming language
MATLAB (R2016a), but also works with older versions of MATLAB (e.g. R2010a)

Additional system requirements
Disk space: 1.98 MB

Dependencies
The executable file (`FlowRateOptimizer.exe`) can run without any other additional files. Optional is to download the image file `splash.png` in order to have a splash screen prior to the start of the application. Both files have to be in the same folder.

For running the `.m` file in MATLAB, make sure the corresponding `.m` and the `.fig` file can both be found by MATLAB.

List of contributors
N/A.

Software location
Archive figshare
   Name: Flow rates in liquid chromatography, gas chromatography and supercritical fluid chromatography: A tool for optimization
   Persistent identifier: https://dx.doi.org/10.6084/m9.figshare.3102247.v1
   Licence: MIT
   Publisher: Joris Meurs
   Version published: v1
   Date published: 10/03/16

Code repository GitHub

Name: Source code for a graphical user interface (GUI) which can be used for optimizing flow rates in liquid chromatography (LC), gas chromatography (GC) and supercritical fluid chromatography (SFC)

Persistent identifier: `https://github.com/jorismeurs/LC_GC_SFC_optimization`

Licence: MIT

Date published: 10/03/16

Support mechanism: GitHub Issues

Language

English.

(3) Reuse potential

This application offers fast and easy calculation for the flow rate of the mobile phase in chromatographic systems under certain conditions, such as particle size, diffusion coefficients and dispersion factors. In this way, system optimization can be done easily. To make the handling of the application even more easy, a brief tutorial is available in the code repository. This tutorial shows which parameters have to be filled out. In addition, figures are shown to support the tutorial text. Additionally, this tool can be integrated to existing chemistry-based calculation tools, for example Calc98 or OpenChrom. Furthermore, the application does not need any pre-installation which means the executable file can be launched directly. However, a standalone application as only available for Windows. In the future, it will be aimed to generate also a standalone application for Linux as well as Mac OS X. Nonetheless, this application is a useful tool for optimizing chromatographic systems which can finds its use in the field of analytical chemistry.

Competing Interests

The author declares that they have no competing interests.

References

1. **Saint-Marcoux, F, Lachatre, G** and **Marquet, P** 2003 Evaluation of an improved general unknown screening procedure using liquid-chromatography-electrospray-mass spectrometry by comparison with gas chromatography and high-performance liquid-chromatographydiode array detection. *Journal of the american society for mass spectrometry*, 14(1): 14–22. DOI: http://dx.doi.org/10.1016/S1044-0305(02)00801-2

2. **Barwick, V J** 1999 Sources of uncertainty in gas chromatography and high-performance liquid chromatography. *Journal of Chromatography A*, 849(1): 13–33. DOI: http://dx.doi.org/10.1016/S0021-9673(99)00537-3

3. **Van Deemter, J J, Zuiderweg, F J** and **van Klinkenberg, A** 1956 Longitudinal diffusion and resistance to mass transfer as causes of nonideality in chromatography. *Chemical Engineering Science*, 5(6): 271–289. DOI: http://dx.doi.org/10.1016/0009-2509(96)81813-6

4. **Done, J N** and **Knox, J H** 1972 The performance of packings in high speed liquid chromatography ii. zipax@ the effect of particle size. *Journal of Chromatographic Science*, 10(10): 606–612. DOI: http://dx.doi.org/10.1093/chromsci/10.10.606

5. **Giddings, J C** 1961 Lateral diffusion and local non-equilibrium in gas chromatography. *Journal of Chromatography A*, 5: 61–67, 1961. DOI: http://dx.doi.org/10.1016/S0021-9673(01)92816-X

6. **Carr, P W** and **Sun, L** 1998 An approximate expression for the minimum plate height produced by knox's equation. *Journal of Microcolumn Separations*, 10(1): 149–152. DOI: http://dx.doi.org/10.1002/(SICI)1520-667X(1998)10:1(149::AID-MCS19)3.0.CO;2-Z

7. **Golay, M J E** 1968 Height equivalent to a thoretical plate of an open tubular column lined with a porous layer. *Analytical Chemistry*, 40(2): 382–384. DOI: http://dx.doi.org/10.1021/ac60258a034

8. **Holler, F J, Skoog, D A** and **Crouch, S R** 2007 *Principles of instrumental analysis*. ISBN 978-0-495-01201-6.

9. **Snyder, L R, Kirkland, J J** and **Dolan, J W** 2011 *Introduction to modern liquid chromatography*. John Wiley & Sons, 2011. ISBN 978-0-470-16754-0.

10. **Dettmer-Wilde, K** and **Engewald, W** 2014 *Practical Gas Chromatography: A Comprehensive Reference*. Springer. DOI: http://dx.doi.org/10.1007/978-3-642-54640-2

11. **Knox, J H** 2002 Band dispersion in chromatographya universal expression for the contribution from the mobile zone. *Journal of Chromatography A*, 960(1): 7–18. DOI: http://dx.doi.org/10.1016/S0021-9673(02)00240-6

12. **Schoenmakers, P J** 1988 Supercritical-fluid chromatography: Open columns vs packed columns. *Journal of High Resolution Chromatography*, 11(3): 278–282. DOI: http://dx.doi.org/10.1002/jhrc.1240110311

13. **Knauer** n.d. How do I select a suitable flow rate according to the column inner diameter? Available at: http://www.knauer.net/en/faq/detail/627-how-do-i-select-a-suitable-flow-rate-according-to-the-column-inner-diamete (Accessed on 2-6-2016).