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.

**Keywords:** Gas Chromatography; Liquid Chromatography; Supercritical Fluid Chromatography; Optimization; Flow rate

**(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 Knox’ 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]:

\[ H = 2 \lambda d_p + \frac{2 \gamma D_m}{u} + \left( \frac{f (k) d^2_p}{D} + \frac{f (k) d^2_m}{D_m} \right) u \]  

(1)

Which is often reduced to:

\[ H = Ad_p + \frac{BD_m}{u} + \frac{Cud^2_p}{D_m} \]  

(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 ($D_f$) 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 $\mathrm{d}H/\mathrm{d}u = 0$. Differentiation of Equation 2 resulted in:

$$\frac{\partial H}{\partial u} = \frac{Cd_p^2}{D_m} \cdot \frac{BD_m}{u^2}$$

(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:

$$u = \frac{Z + D_f H - AD_m d_p^2}{2Cd_p^2} - \frac{Z - D_m H + AD_m d_p^2}{2Cd_p^2}$$

(4)

where,

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

(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]:

$$h = A + \frac{B}{u} + Cv$$

(6)

The reduced plate height ($h$) is defined by respectively the A-term, B-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]:

$$A = 2\lambda d_p$$

(7)

$$B = 2\gamma D_c$$

(8)

$$C = \frac{8k}{\pi^2} \frac{d_l^2}{D_f}$$

(9)

Where, $\lambda$ is the correction factor for the irregularity of the column packing, $d_p$ is the particle size, $\gamma$ is the labyrinth factor (value between 0 and 1), $D_c$ 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 $D_f$ 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]:

$$h = AAv + \frac{B}{u^2} + Cv$$

(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]:

$$H = \frac{2D_m}{u} + f(k)\frac{d^2_p}{D_m} + g(k)\frac{d^2_l}{D_f}$$

(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, $d_p$ is the diameter of the column and $d_l$ is the film thickness. Further, $f$ and $g$ are functions of the retention factor ($k$). These functions are described below [12]:

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

(12)

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

(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:

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

(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 $10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$, $10^{-6} \text{ m}^2 \cdot \text{s}^{-1}$ and $10^{-8} \text{ m}^2 \cdot \text{s}^{-1}$ 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 (linearpace) 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
height is determined. After, the corresponding value for the linear velocity can be determined. This value is converted to the optimal flow rate in m$^3$/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, 2.369·10$^{-9}$ m$^2$/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 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

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| 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 – 4.6               | 0.80 – 2.0                  | 1.11                          |

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

Table 1: Overview of units per variable.

| Type of column | Variable | Unit |
|----------------|----------|------|
| Packed column  | A        | Dimensionless |
|                | B        | Dimensionless |
|                | C        | Dimensionless |
|                | D$_n$    | m$^2$/s$^{-1}$ |
|                | u        | m$/s$ |
|                | d$_p$    | µm |
|                | k        | Dimensionless |

| Capillary column | d$_c$ | mm |
|------------------|-------|----|
|                  | D$_f$ | m$^2$/s$^{-1}$ |
|                  | u     | m$/s$ |
|                  | d$_f$ | µm |
|                  | k     | Dimensionless |

| 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].
(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, dispersion coefficients and diffusion 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.

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