



Metrics of separation performance in chromatography

Part 3: General separation performance of linear solvent strength gradient liquid chromatography



Leonid M. Blumberg^{a,*}, Gert Desmet^b

^a Advachrom, P.O. Box 1243, Wilmington, DE 19801, USA

^b Department of Chemical Engineering, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium

ARTICLE INFO

Article history:

Received 6 May 2015

Received in revised form 27 July 2015

Accepted 31 July 2015

Available online 5 August 2015

Keywords:

Peak capacity

Separation capacity

Separability

Utilization of separability

Speed of analysis

Mixing rate

Characteristic solvent strength

Characteristic strength-constant

ABSTRACT

The separation performance metrics defined in Part 1 of this series are applied to the evaluation of general separation performance of linear solvent strength (LSS) gradient LC. Among the evaluated metrics was the peak capacity of an arbitrary segment of a chromatogram. Also evaluated were the peak width, the separability of two solutes, the utilization of separability, and the speed of analysis—all at an arbitrary point of a chromatogram. The means are provided to express all these metrics as functions of an arbitrary time during LC analysis, as functions of an arbitrary outlet solvent strength changing during the analysis, as functions of parameters of the solutes eluting during the analysis, and as functions of several other factors. The separation performance of gradient LC is compared with the separation performance of temperature-programmed GC evaluated in Part 2.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

This report continues the study of metrics of *general* separation performance in chromatography started in Parts 1 and 2 of this series [1,2]. Part 1 described the definitions and application of the metrics to *static* analyses (isothermal isobaric GC, isocratic isothermal isobaric LC, etc.). In Part 2, the metrics were applied to evaluation of general separation performance of temperature-programmed GC. This report considers the application of the same metrics to evaluation of general separation performance of LSS (linear solvent strength) gradient LC of arbitrary samples. However, only the analyses of the small-molecule mixtures (molecular weight 100–500) are considered in numerical examples. As in Parts 1 and 2, the term *separation performance* infers here the **general separation performance** (such as the peak capacity of the entire analysis or of its part, etc., rather than, say, separation of particular solutes). In addition to its reliance on Parts 1 and 2, the study in this report relies on the earlier published summary [3,4] of the properties of LSS gradient LC. Although many (but not all) those properties were known [5–9] prior to publication of the summary

[3,4], the latter provided the most extensive set of equations in a single source based on a single framework.

The LSS model offers only an approximate description of the real gradient LC techniques such as RPLC (reverse-phase LC) and others [5–7,9]. However, the advantage of the LSS model is that it frequently leads to close-form solutions offering valuable insight into the factors affecting performance of gradient LC and serving as the baseline for the studies of more accurate (but usually more complex) models. As its title suggests, this report is only concerned with LSS *gradient* LC. In view of that, the term *gradient* LC is treated below as a synonym of the term **LSS gradient LC**.

In many ways, gradient LC is similar to temperature-programmed GC. Both are *dynamic* [3,10] (*time-varying* [11]) techniques where, during their migration along the column, the solutes accelerate relative to the mobile phase velocity. In both cases, the acceleration comes from programmable reduction in the solute retention factors and leads to substantial (order of magnitude or more) peak width reduction compared to the static counterparts of the respective techniques.

However, there is a substantial difference in activation of the solute acceleration in the two techniques. In temperature-programmed GC, the acceleration is caused by programmable increase in the column temperature (T) which is *uniform* along the

* Corresponding author. Tel.: +1 302 888 1113.

E-mail address: leon@advachrom.com (L.M. Blumberg).

column (at any given time, T is the same at any location along the column) [10–12]. Let ϕ be the volume fraction of a stronger solvent in the mobile phase also interpreted in this report as the solvent strength. The solute acceleration in gradient LC is caused by programmable increase in the inlet solvent strength (ϕ_i). The stronger solvent travels from the inlet toward the outlet with the solvent flow. As a result, the solvent strength (ϕ) is not uniform along a column (at any given time, ϕ declines toward the outlet) [3–7]. The uniform T in temperature-programmed GC, and the non-uniform ϕ in gradient LC constitute an important difference between the two techniques [4].

To get a better insight into the effect of the gradients on performance of gradient LC, it is important to distinguish between the fact of a solute acceleration existing in dynamic GC and LC, and the fact, existing only in gradient LC, that the solvent strength in the column is not uniform. In this report, the temporal rates of changes of chromatographic parameters in time are distinguished from spatial gradients representing only the changes with distance along a column. The temporal rate, $R_T = dT/dt$, of the increase in T with time (t) in temperature-programmed GC is the heating rate. The temporal rate [3,7,13], of the increase in ϕ_i can be called as the mixing rate—a term that appears to be reasonably descriptive, short, and analogous to the heating rate in GC considered in Part 2 of this series. The solvent strength gradient, $g_\phi = \partial\phi / \partial z$, is a measure of the steepness [5] of the spatial decline in non-uniform solvent strength with the longitudinal (along the column) distance (z) from the column inlet.

The negative solvent strength gradient creates a positive gradient of the retention factor and a negative gradient of velocity within each solute band (zone). As a result, the front of a solute band travels slower than its rear causing the band compression (narrowing, sharpening) that can sharpen the bands down to almost half, in some cases, of the width that they would have under uniform but otherwise identical conditions [4,5,8,9,14].

The band compression phenomenon described by Snyder and Saunders [15] in 1969 and theoretically quantified by Poppe et al. [8] in 1981 is a reasonably transparent and well understood phenomenon. It should be recognized, however, that the spatial widths of the solute bands are not of a primary importance for the column performance. The temporal widths (in units of time) of the peaks in chromatograms are. The distinction between a solute band within a column (briefly, the band) and its spatial width (briefly, the band width) on the one hand, and a peak in a chromatogram (briefly, the peak) and its temporal width (briefly, the peak width) on the other hand is recognized throughout this report. As the peak widths, and not the band widths, are the factors that directly affect the column performance, a study [3–9,11,14,16] of evolution and formation of a solute band during its migration in LC column can be considered as no more than an intermediary step in finding the effect of the gradients on the peak widths and retention times.

As mentioned earlier, the factor affecting substantial peak width reduction in dynamic techniques compared to their static counterparts is the solute acceleration in dynamic techniques. It is frequently assumed that the band compression in gradient LC causes an additional peak sharpening known as peak focusing [5,9,14,17], and a peak capacity increase [9,17]. This is not necessarily the case [4] because the negative solvent strength gradients not only compress the solute bands, but also slow down their elution [4]. The latter tends to increase the peak widths thus partially compensating or overcompensating for the peak narrowing due to the band compression. The net effect of the typical velocity gradients can be the peak focusing or defocusing (broadening) [4]. These considerations suggest that accounting for one effect of the gradient while neglecting the other might lead to substantial exaggeration of the impact of the gradient. Thus, disregarding the gradient-caused slowing of the solute elution while accounting for the band compression leads to exaggerated expectation of the peak focusing and

the peak capacity increase [9,17]. On the other hand, disregarding the gradient band compression while taking full account of all other effects of the gradient led [18] to substantially undervalued peak capacity under typical conditions. To avoid these pitfalls, this report took full account of all effects of the gradients on all evaluated parameters. This led to a different approach to the treatment of the metrics of separation performance in this report compared to the treatment of temperature-programmed GC in Part 2. Thus, the study of a column performance in Part 2 essentially included only the highly interactive solutes (the ones that are strongly retained at the beginning of a heating ramp). To account for the gradients, this report includes all solutes regardless of their initial retention.

The key components of a column separation performance are the peak retention times and widths. The mathematical expressions describing these parameters are known from literature [3–9]. However, the known equations describe the peak parameters as functions of typically unknown initial properties of the solutes (like the initial retention factor, $k_{i,init}$) at the beginning of the gradient run. This substantially complicates theoretical prediction of the metrics of a column performance (such as the peak capacity) from the experimental conditions.

An approximate expression of the peak capacity of gradient analysis as a function of its time (t) is known from Neue [18]. However, the expression does not account for the gradient band compression thus significantly underestimating the peak capacity.

In this report, we asked and answered the following key questions. What peak capacity is accumulated in gradient LC analysis by the time when its outlet solvent strength (ϕ_o) reaches an arbitrarily chosen level? What are the values of other performance metrics of interest at that time? In answering these questions, the study in the report accounts for all factors affecting the performance of LSS gradient LC, including the gradient band compression. The report also provides dependencies of ϕ_o on several alternative variables of possible interest such as the arbitrary t , $k_{i,init}$, and others. Substitution of these dependencies in an expression of a metric as a function of ϕ_o transforms that metric into a function of a chosen alternative variable. As in Parts 1 and 2, the constraints introduced here are highlighted by the **bold face** type.

2. Experimental

The theoretical results in this report were illustrated by the properties of an ordinary LC analysis—one of many published by LC column manufacturers. The following column and solvent flow rate were chosen for the forthcoming numerical data. Column ($L \times d_c \times d_p$): 100 mm \times 4.6 mm \times 3 μ m. Solvent flow: 1 mL/min. The linearly programmed fraction (ϕ_i) of a stronger solvent in a weaker one at the column inlet: 15% @ 0 min, 85% at 17.5 min—the 70% increase ($\Delta\phi_{i,max}$) in the solvent composition occurred during 17.5 min time interval (t_C). The hold-up volume of the column was estimated as [5] 1 mL yielding 1 min hold-up time (t_0). It is assumed that the solvent flow was close to optimal and the dimensionless plate height (h) [19] was 2. These parameters are compiled in Table 1. They were used below only in numerical examples, but not in general equations.

3. Theory and discussion

The results of Parts 1 and 2 are frequently used here without additional explanations. To simplify the references to these results,

Table 1
Experimental parameters in numerical examples.

Parameter	L (mm)	d_p (μ m)	t_0 (min)	h	$\Delta\phi_{i,max}$	t_C (min)
Value	100	3	1	2	0.7	17.5

Download English Version:

<https://daneshyari.com/en/article/1201168>

Download Persian Version:

<https://daneshyari.com/article/1201168>

[Daneshyari.com](https://daneshyari.com)