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Optimal mixing rate in linear solvent strength gradient liquid chromatography. Balanced mixing program

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ABSTRACT

The *mixing rate* (R_ϕ) is the temporal rate of increase in the solvent strength in gradient LC. The *optimal* R_ϕ ($R_{\phi,\mathrm{Opt}}$) is the one at which a required peak capacity of gradient LC analysis is obtained in the shortest time. The *balanced* mixing program is a one where, for better separation of early eluting solutes, the mixing ramp is preceded by a balanced isocratic hold of the duration depending on R_ϕ. The improvement in the separation of the earlier eluites due to the balanced programming has been evaluated. The value of $R_{\phi,\mathrm{Opt}}$ depends on the solvent composition range covered by the mixing ramp and on the column pressure conditions. The $R_{\phi,\mathrm{Opt}}$ for a column operating at maximum instrumental pressure is different from $R_{\phi,\mathrm{Opt}}$ for a column operating below the instrumental pressure limit. On the other hand, it has been shown that the difference in the $R_{\phi,\mathrm{Opt}}$ values under different conditions is not very large so that a single default R_ϕ previously recommended for gradient analyses without the isocratic hold also yields a good approximation to the shortest analysis time for all conditions in the balanced analyses. With or without the initial balance isocratic hold, the recommended default R_ϕ is about $5\%/t_0$ (5% increase in the solvent strength per each t_0-long increment in time) for small-molecule samples, and about an order of magnitude slower ($0.5\%/t_0$) for protein samples. A discussion illustrating the use of the optimization criteria employed here for the techniques other than LSS gradient LC is included.

1. Introduction

To a large degree, this report continues our previous study [1] of the mixing rate optimization in LSS (linear solvent strength) gradient LC. The *mixing rate* [1,2] is the temporal rate, $R_\phi = \partial\phi_i/\partial t$, of programmable change in the solvent strength (ϕ_i) – the volume-fraction of stronger solvent in the mobile phase – at the column inlet.

Many studies of optimization of several performance factors of gradient LC were published [1–11]. The purpose of this report and its predecessor [1] was to find the *optimal mixing rate* ($R_{\phi,\mathrm{Opt}}$) defined [1] as the **constant** R_ϕ at which a required *peak capacity* [12–15] of LC analysis and, more specifically, a required *separation capacity* [2,14–16] of LC column are obtained in the shortest time. As before [1], only the LSS (linear solvent strength) gradient LC [17–22] is considered in this report, and the terms *gradient LC* and *LSS gradient LC* are treated here as **synonyms**.

In a previous study [1], we found $R_{\phi,\mathrm{Opt}}$ for what can be called as the *no-hold mixing* program – a single-ramp program where the *mixing ramp* at the column inlet starts simultaneously with the sample introduction. If a sample in such analysis contains nearly identical solutes [2] that are slightly retained at the *initial* solvent strength (ϕ_{init}) of the *mixing ramp* [1,2], further increase in the solvent strength (ϕ) during migration of these solutes will then further degrade their retention and the *separation* [14,15,23]. This degradation can be reduced by inserting an *isocratic hold* of strength ϕ_{init} prior to the mixing ramp. The duration of the hold can be chosen in such a way that all solutes eluting during the ramp following the hold elute with the same retention factor as the retention factor at the end of the hold. Due to analogy of such program to that of the *balanced heating program* in temperature-programmed GC [23,24], we call this mixing program and its components (the hold, the ramp) as the *balanced* ones.

The method of finding $R_{\phi,\mathrm{Opt}}$ in this report is substantially similar to the one earlier employed for the mixing rate optimization in no-hold mixing in gradient LC [1], and for the heating rate optimization in no-hold heating in temperature-programmed GC [16,25]. Only the key points of the optimization method are reproduced here. A reader interested in additional details can find them in pre-

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Nomenclature

Subscripts

def	Recommended default value
i	Inlet parameter
init	Value at the beginning of the mixing ramp
o	Parameter measured at the column outlet
opt	Optimum at lowest H
Opt	Optimum at shortest t at fixed s
0	Parameter of unretained solute

Symbols

g	Normalized range of a sample, Eq. (10)
g_o	Normalized range of mixing ramp, Eq. (10)
i	System order (comments to Eq. (5))
k	Retention factor
L	Column length
M	Molecular weight
n	Peak capacity, Eq. (1)
R_ϕ	Mixing rate
r_ϕ	Dimensionless mixing rate, Eq. (13)
s	Separation capacity (number of σ-wide segments in the separation space)
t	Time of analysis
t_0	Void time
t_c	Parameter defined in Eq. (5)
U_g	Utilization of separability, Fig. 5
u_0	Solvent velocity, Eq. (6)
σ	Peak standard deviation
Φ_{char}	Characteristic strength-constant
ϕ	Solvent strength
ϕ_{char}	Characteristic solvent strength
σ_a	Asymptotic level of σ during the ramp, Eq. (24)

vious publication [1]. Unless it is explicitly stated otherwise, the *standard deviation* (σ) of a peak [26,27] is the only peak *width* metric in this report. The use of this metric in this report makes its results applicable to the peaks of any (not necessarily *Gaussian*) shape. As in the preceding paragraphs, the constraints introduced below are highlighted by the **bold face** type.

2. Theory

2.1. Known relevant results

2.1.1. Peak capacity and separation capacity

The *separation capacity* [2,14–16] (s) of a column in a chromatographic analysis is the number of σ-*slots* (σ-wide segments) in the separation space of the analysis. If necessary, the *peak capacity* [12–15] (n) of a chromatographic analysis can be found from s as [2,14,15]:

$$n = \frac{s}{\Delta s_{min}} \tag{1}$$

where Δs_{min} is the smallest number of σ-slots between two neighboring peaks required for *resolving* (quantifiably and identifiably separating) the peaks. Quantity Δs_{min} depends on the ability of data analysis sub-system to resolve poorly separated peaks [14,15], and on other factors external to the column. The Δs_{min} also depends on the peak shapes. As in previous studies [1,2,14–16,23,24] of a column performance and its optimization, metric s, being independent of the factors external to the column and of the peak shapes, is more suitable than n for the study in this report.

2.1.2. Column efficiency and void time

An important parameter of a column performance is its *efficiency* [1,2,14,16,23] (E) defined for isocratic conditions as [26]:

$$E = \frac{t}{\sigma}, \quad \text{(isocratic conditions)} \tag{2}$$

where t and σ are the *peak's retention time* and width (standard deviation), respectively. In many respects, E is a better metric [2,14,16,23,24] than the wider known *plate number*, $N = E^2$.

The E of a solute in gradient LC can be defined as the one *isocratically* measured for the solute according to Eq. (2) under conditions reflecting the solvent composition experienced by the solute during its gradient migration. So measured E can be different for different solutes as they reside in the column during the gradient analysis for different times during which the solvent strength changes. As a result, E in a gradient analysis can be a time-dependent quantity. Typically, however, the difference between the values of E for the solutes in the same analysis is practically insignificant and can be ignored for the sake of simplicity [1,2,8,17,21,28,29]. It is assumed below that E remains **fixed** during a gradient analysis. In this study, the assumption led to the closed-form solutions providing valuable general insights into the factors affecting the optimal mixing rate in gradient LC. The assumption of a fixed E in a gradient analysis implies that the plate height:

$$H = \frac{L}{E^2} \tag{3}$$

in a L-long column is also fixed during the gradient analysis.

The void time (t_0) in LSS gradient LC does not change during the analysis [17,22]. The relationship between E and t_0 can be summarized as [1,30]:

$$t_0 = t_c E^i, \quad i = (2,\ 4) \tag{4}$$

$$t_c = \begin{cases} H/u_0, & i = 2 \\ (H^2/B_o)(\eta/\Delta p), & i = 4 \end{cases} \tag{5}$$

where $i = 4$ (the *quartic*, the *fourth order* system) represents a column operating at the *maximum instrumental pressure* (the highest pressure available from LC instrument) and requiring a simultaneous increase in the particle size and in the column length for increasing the column efficiency. The case of $i = 2$ (the *quadratic*, the *second order* system) represents a column operating at *submaximum* pressure (below the maximum instrumental pressure) for which an increase in the column length alone is sufficient for increasing the column efficiency. The scaling factor (t_c) in Eqs. (4) and (5) is measured in units of time and depends on the system order (i) and on the operational parameters which might include the column *permeability* (B_o), *pressure drop* (Δp), the solvent *viscosity* (η) and its *velocity*:

$$u_0 = \frac{L}{t_0} \tag{6}$$

2.1.3. Retention

As stated earlier, the terms *gradient LC* and *LSS gradient LC* are treated in this report as synonyms. Let ϕ and k be, respectively, the solvent *strength* (the volume-fraction of the strongest solvent in the mobile phase) and the solute *retention factor*. The linear dependence of $\ln k$ on ϕ can be expressed as [2,22]:

$$\ln k = \ln k(\phi) = \frac{\phi_{char} - \phi}{\Phi_{char}} \tag{7}$$

where ϕ_{char} and Φ_{char} are the *characteristic solvent strength* and the *characteristic strength-constant* of the solute. The properties and advantages of these parameters over the more customary parameters [17] k_w and S introduced by Snyder are described elsewhere