Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Peak capacity and peak capacity per unit time in capillary and microchip zone electrophoresis

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ARTICLE INFO

Article history:
Received 5 May 2017
Received in revised form 11 August 2017
Accepted 14 August 2017
Available online xxx

Keywords:
Peak capacity
Peak capacity per unit time
Capillary electrophoresis
Microchip electrophoresis

ABSTRACT

The origins of the peak capacity concept are described and the important contributions to the development of that concept in chromatography and electrophoresis are reviewed. Whereas numerous quantitative expressions have been reported for one- and two-dimensional separations, most are focused on chromatographic separations and few, if any, quantitative unbiased expressions have been developed for capillary or microchip zone electrophoresis. Making the common assumption that longitudinal diffusion is the predominant source of zone broadening in capillary electrophoresis, analytical expressions for the peak capacity are derived, first in terms of migration time, diffusion coefficient, migration distance, and desired resolution, and then in terms of the remaining underlying fundamental parameters (electric field, electroosmotic and electrophoretic mobilities) that determine the migration time. The latter expressions clearly illustrate the direct square root dependence of peak capacity on electric field and migration distance and the inverse square root dependence on solute diffusion coefficient. Conditions that result in a high peak capacity will result in a low peak capacity per unit time and vice-versa. For a given symmetrical range of relative electrophoretic mobilities for co- and counter-electroosmotic species (cations and anions), the peak capacity increases with the square root of the electric field even as the temporal window narrows considerably, resulting in a significant reduction in analysis time. Over a broad relative electrophoretic mobility interval [-0.9, 0.9], an approximately two-fold greater amount of peak capacity can be generated for counter-electroosmotic species although it takes about five-fold longer to do so, consistent with the well-known bias in migration time and resolving power for co- and counter-electroosmotic species. The optimum lower bound of the relative electrophoretic mobility interval [$\mu_{r,Z}$, $\mu_{r,A}$] that provides the maximum peak capacity per unit time is a simple function of the upper bound, but its direct application is limited to samples with analytes whose electrophoretic mobilities can be varied independently of electroosmotic flow. For samples containing both co- and counter-electroosmotic ions whose electrophoretic mobilities cannot be easily manipulated, comparable levels of peak capacity and peak capacity per unit time for all ions can be obtained by adjusting the EOF to devote the same amount of time to the separation of each class of ions; this corresponds to $\mu_{r,Z} = -0.5$.

1. Introduction

First introduced by Giddings in 1967 for size exclusion chromatography (SEC) and other isocratic elution methods [1] and then applied by Horvath and Lipsky to gradient elution later in the same year [2], the concept of peak capacity or "the maximum number of peaks ... [that can] be separated on a given column" has become a very important metric in separation science. As noted by Neue [3], in contrast to the classical plate number, which is typically only measured using a single compound in isocratic elution and is not directly applicable to gradient elution without some adjustments, the peak capacity is "a measure of the separation power that includes the entire chromatogram together with the variability of the peak width over the chromatogram." Although the theoretical treatment can potentially be complex, peak capacity is equally applicable to many types of separations, not just isocratic or gradient elution liquid chromatography and isothermal or programmed temperature gas chromatography. It is a key parameter in a well-developed statistical model of overlap (SMO) for one-dimensional [4] and two-dimensional separations [5] as well as recent stochastic

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http://dx.doi.org/10.1016/j.chroma.2017.08.043
0021-9673/

simulations [6] that determines among other things, in conjunction with the number of components in a sample, the distinct probabilities that (i) a given sample component will be separated from all other components and (ii) all of the sample components will be separated from each other.

The concept of peak capacity has not received nearly as much attention or been as fully developed in capillary zone electrophoresis (CZE) as it has in gas and liquid chromatography. For purposes of context it is constructive to briefly review the key results obtained for various types of chromatography.

Giddings' expression for peak capacity in SEC and other isocratic elution methods was

$$n = 1 + \frac{\sqrt{N}}{m} \ln\left(\frac{V_n}{V_1}\right) \quad (1)$$

where n is the peak capacity, m is the peak width (in multiples of σ), N is the number of theoretical plates, and V_1 and V_n are the retention volumes of the first and n^{th} peaks, respectively [1]. In this definition, the ratio of peak width to retention volume is assumed to be the same for all peaks, i.e., a constant plate number that is typically assumed for isocratic elution. Also, the value of m defines the desired resolution (e.g., m = 4 or 6 implies R_s (resolution) = 1 or 1.5, respectively). In later work, Giddings replaced m with $4\sigma R_s$ and used n_c rather than n to symbolize peak capacity [7].

Horváth and Lipsky's expression for the peak capacity of a separation, where the peak width is constant (or nearly so) throughout the separation, i.e., liquid chromatography with gradient elution or temperature programmed gas chromatography, was

$$n = \frac{\sqrt{N}}{4}\left(\frac{V_n}{V_1} - 1\right) \quad (2)$$

where N was measured independently for the first peak under isocratic or isothermal conditions [2].

Grushka utilized two different approaches based on Giddings' work and in 1970 reported the following result for the peak capacity in isocratic elution in terms of the retention times of the first and nth peaks, where the first peak was assumed to be unretained (t_0) [8].

$$n = 1 + \frac{\sqrt{N}}{4}\ln\left(\frac{t_n}{t_0}\right) \quad (3)$$

Because the maximum retention factor in a separation window, k_{max}, can be expressed as $t_{last}/t_0 - 1$, Grushka's isocratic peak capacity equation has sometimes been written as

$$n = 1 + \frac{\sqrt{N}}{4}\ln(1 + k_{max}) \quad (4)$$

In the same paper Grushka also reported a commonly used expression for peak capacity under gradient elution (LC) or temperature programmed (GC) conditions [8]:

$$n = 1 + \frac{t_n - t_0}{W} \quad (5)$$

where W is the 4σ baseline width, assumed to be constant and thus independent of retention time.

Capillary zone electrophoresis (CZE), an analytical technique used to separate samples into their individual components based on their differential migration in a capillary under an applied electric field, was pioneered by Mikkers et al. [9,10] and Jorgenson and Lukacs [11–13] as an alternative liquid-phase separation technique to HPLC. This research captured the attention of the separation science community as the benefits of small capillaries (200 μm i.d. PTFE capillaries by Mikkers et al. and 75 μm glass capillaries by Jorgenson and Lukacs) were quickly realized, including the essential negation of the detrimental effect of convection by both groups and the very narrow zones (N > 400,000) achieved using the smaller i.d. glass capillaries. Therefore, CZE analysis could be carried out in free solution without the need for supporting separation media as in HPLC.

While numerous reports have been published on the enhanced efficiency of CZE separations compared to traditional liquid chromatography separations, few papers have examined peak capacity in CZE from a theoretical perspective. For context we describe two relatively early contributions to the concept of peak capacity in voltage-driven separation techniques related to CZE before proceeding to early, representative applications of peak capacity in the latter.

Giddings considered plate height and plate number in sedimentation and in a non-capillary electrophoresis format in order to develop expressions for peak capacity that could be related to those deduced for liquid chromatography [14]. Three scenarios were considered, in which separations were achieved by differences in (i) charge for similar size molecules, (ii) frictional drag (for molecules of the same charge), and (iii) charge for molecules with a gradually increasing charge with increasing size. The results [14] are shown below

$$n = \sqrt{\frac{-\Delta\mu^o_{max}}{32RT}}(i);\ n = \sqrt{\frac{-\Delta\mu^o_{max}}{8RT}}(ii);\ n = \sqrt{\frac{-\Delta\mu^o}{32RT}}\ln\frac{L}{L_0} = \frac{\sqrt{N}}{4}\ln\frac{L}{L_0}(iii) \quad (6)$$

where $\Delta\mu^\circ$ is the chemical potential drop of the species in migrating a specified distance, R is the ideal gas constant, T is absolute temperature, L is the total migration distance, and L_0 is the minimum migration distance from the starting point below which the initial sample width is no longer negligible. According to Giddings, a reasonable approximation to all of the above results is

$$n \approx \frac{\sqrt{N_{max}}}{2} \quad (7)$$

Terabe et al. adapted the results of Giddings and Grushka for isocratic liquid chromatography (Eqs. (1) and (3)) to micellar electrokinetic chromatography (MEKC)

$$n = 1 + \frac{\sqrt{N}}{4}\ln\left(\frac{t_{mc}}{t_0}\right) \quad (8)$$

where t_{mc} is the migration time of the micelle.

Within the field of CZE, Jones and Jandik utilized Eq. (1) (Giddings) to compare the peak capacity of ion chromatography and CZE, using an average plate count for the 30 anions separated by CZE whose peak widths were increasing significantly [15]. Similarly, Nashabeh and El Rassi used the Giddings/Grushka equations for the measurement of peak capacity in a CZE-mediated separation of proteins,

$$n = 1 + \frac{\sqrt{N_{av}}}{4}\ln\left(\frac{t_f}{t_i}\right) \quad (9)$$

where N_{av} is the average plate number for their co-electroosmotic analyte proteins, and t_i and t_f are the migration times of the first-eluting protein lysozyme and the last-eluting neutral marker phenol, respectively [16]. Likewise, Poli and Schure utilized the average number of theoretical plates in combination with Eq. (1) to estimate the effective peak capacity for the separation of some poly(styrenesulfonates),

$$n_c = 1 + \frac{\sqrt{N_{av}}}{4R_s}\ln\left(\frac{t_{max}}{t_{min}}\right) \quad (10)$$

where R_s is the resolution [17]. Although in general later studies that sought to estimate or measure the peak capacity in CZE have utilized one of the above approaches developed for chromatography, a notable exception was the automated approach by Lan and Jorgenson to measure peak widths and ultimately the peak capacity in complex separations [18].

Please cite this article in press as: J.P. Foley, et al., Peak capacity and peak capacity per unit time in capillary and microchip zone electrophoresis, J. Chromatogr. A (2017), http://dx.doi.org/10.1016/j.chroma.2017.08.043