



Kinetic performance evaluation and perspectives of contemporary packed column capillary electrochromatography[☆]



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ABSTRACT

Capillary electrochromatography (CEC) is in essence a highly efficient and fast separation technique but practical constraints limit the current performance, robustness and routine implementation of the technique. In this work the kinetic performance limit (KPL) curve was used to evaluate commercial packed column CEC; this firstly in order to assess the broader applicability of the kinetic plot approach in electrodriven chromatographic techniques, and secondly to allow a more general unbiased comparison with HPLC performance. Evaluations were performed with a mixture of well retained and electrophoretically neutral phenones, to allow the observation of only chromatographic processes. Initial CEC retention time irreproducibility issues were solved by applying high acetonitrile content (80%) in the mobile phase, and solute retention was increased by increasing the phenone chain length. Comparison was performed with HPLC, with a column packed with an identical stationary phase to allow measurement of the performance under optimal conditions, and not with μ -LC on the CEC column as extra column peak broadening phenomena would thereby negatively affect the μ -LC performance. This comparison demonstrated that current HPLC performance largely outcompetes what is achievable with contemporary packed column CEC. Interestingly, significantly improved CEC performance could be obtained at lower temperatures (10 °C) indicating a persistent degree of joule heating phenomena taking place in the contemporary packed column (100 μ m) CEC approach. Effective suppression of the latter opens possibilities for increasing the applicable voltage and outperforming HPLC and UHPLC.

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1. Introduction

Since the implementation of packed column capillary electrochromatography (CEC) for the analysis of neutral solutes in the early nineties, the technique has enjoyed significant interest and has been repeatedly appraised for its superior separation performance compared to the pressure driven analogues [1–3]. In CEC separation between solutes is achieved by either distinctive partitioning between two phases and or by the differences in their respective electrophoretic mobilities. Next to peak focusing due to electro-stacking phenomena of charged solutes, in CEC lower plate heights can be obtained by the plug-like flow profile of the electrodriven flow, which cause less band broadening compared to the parabolic flow profile in pressure driven techniques [1,4–7]. Furthermore, the flow velocity is independent of the particle's size

which permits the use of longer columns and of smaller particles. This has been illustrated for packed column CEC, for example, by Dadoo et al. who reported efficiencies of 750,000 plates with a 30 cm packed bed of 1.5 μ m non-porous particles in a 100 μ m i.d. fused silica capillary [8]; and by Ludtke et al. demonstrating efficiencies of up to 3000,000 plates per metre with an 8.6 cm packed bed of 0.5 μ m C8 particles [9]. Next to the packed CEC columns [10–17], various alternative column formats have been explored in order to address some of the mentioned issues above, including open-tubular [3,18], monolithic [19–21] and pillar array columns [22–24]. Although these are very promising approaches, it appears that thus far none of these alternative column formats underwent sufficient development for commercial implementation.

However, after two decades of CEC development, industrial implementation of CEC remains largely unreported. Although a limited number capillary electrodriven techniques effectively broke through (such as nucleotide sequencing tools [25–27]), the acclaimed advantages of CEC should have resulted in a number genuinely applied methods. The absence thereof can be partially related to inherent problems of capillary separation techniques such as a relatively limited detection sensitivity and challenging

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hyphenation to mass spectrometry [28–30], or to specific CEC related issues such as the absence of commercial instrumentation allowing gradient analysis, method robustness issues and short column life time. A major issue in CEC is also the fact that the generation of flow is coupled to the properties of the particle and therefore selectivity and flow generation cannot be optimized independently. Many of the modern end-capped HPLC packings produce only very low EOF velocities. Although solutions could conceivably be envisaged for the above, according to the authors, a major hurdle of contemporary CEC is that the effective CEC column performance is in many cases only mildly better or comparable to HPLC, and which is quickly becoming obsolete by the advances in UHPLC technology. This stands to contrast to the ultra-high efficiency CEC results described earlier, however, in much of the earlier packed column CEC literature high efficiencies are obtained on columns packed with non-porous particles, or with porous material but under poorly retentive conditions [18,31–35] or by analyzing solutes undergoing electrostatic peak focusing phenomena [17,19,36–37]. Implementation of an independent tool, allowing the least biased evaluation of CEC column performance seems therefore a necessary development.

In liquid chromatography the comparison of columns in terms of achievable efficiency and speed of analysis, has been much facilitated since the introduction of the kinetic performance limit (KPL) method [38]. The resulting kinetic plot (KP) thereby converts the plate height versus linear velocity curve to a speed versus efficiency curve and incorporates column permeability information, as the maximum efficiency is limited by the maximum applicable pressure drop. Consequently the KPL method represents the shortest possible separation time for a given efficiency (measured in plate number N) or the highest possible efficiency for a given separation time for each column. In HPLC the plot can be constructed by implementing the values of the efficiency and the pressure drop, measured at different linear mobile phase velocities (u_0) into the next two equations (Eqs. (1) and (2)) [39]:

$$N = \frac{\Delta P_{\max}}{\eta} \left(\frac{K_{v0}}{u_0 H} \right) \quad (1)$$

$$t_0 = \frac{\Delta P_{\max}}{\eta} \frac{K_{v0}}{u_0^2} \quad (2)$$

where t_0 is the column void time, η is the viscosity, K_{v0} the column permeability and ΔP_{\max} represents the maximum pressure the instrument can provide or the column can endure. As the method assumes a constant plate height at the same linear mobile phase velocities, regardless of the column length, one of the prerequisites is the occurrence of an invariable column permeability irrespectively of the length of the column. A second prerequisite of the KPL method is the invariable retention factor between compared kinetic plots to preserve an identical retention behaviour of the standards. Note that both prerequisites can be detrimentally affected when ultra-high pressures are used, leading to the occurrence of vicious heating, increasing plate heights and decreasing retention. Although the kinetic plot method was originally developed and tested with isocratic analyses on packed columns, the methodology can be also applied for gradient analyses [40,41]. The KPL approach is particularly suitable to compare the performance of columns with broadly differing properties. For example, in this way the maximal achievable performance of open tubular and monolithic formats and of columns packed with various particle sizes and morphologies at various temperatures can be directly compared through a single kinetic plot [42–47]. In pressure driven techniques like LC, the maximum number of plates that can be obtained with a certain mobile phase velocity is determined by the permeability of the column. However, CEC is only limited by the mobility of the electro-osmotic flow (EOF). Thus the practical constraint in CEC is

not the pressure drop but by the potential drop as demonstrated in the following equation:

$$u_0 = \mu_{\text{EOF}} \frac{\Delta V}{L} \quad (3)$$

where μ_{EOF} stands for the electro osmotic flow mobility, ΔV for the applied voltage and L represents the total length of the capillary. Derived from Eq. (3) the kinetic plot in CEC can be described by the next equations (Eqs. (4) and (5)) [48].

$$N = \mu_{\text{EOF}} \frac{\Delta V}{H u_0} \quad (4)$$

$$t_0 = \mu_{\text{EOF}} \frac{\Delta V}{u_0^2} \quad (5)$$

In a similar way as in pressure driven techniques, where the KP is obtained by extrapolation to the maximum deliverable system pressure, can the KP in electrodriven techniques be obtained by extrapolating to the maximum voltage. Unmistakable, the most important prerequisite of the KP approach in CEC comprises an invariable electro-osmotic flow (EOF), independent of the length and position in the capillary. The dependency of the EOF is represented in the following equation:

$$\mu_{\text{EOF}} = \frac{\varepsilon_0 \varepsilon_r \zeta}{\eta} \quad (6)$$

where ε_0 is the permittivity of a vacuum, ε_r is the dielectric constant of the background electrolyte and ζ is the zeta potential [2]. The latter arises from the distribution of counter-ions in a liquid around an ionized surface area, in this case the silica wall of the capillary and the particle surface. The counter-ions will maintain a charge balance and meanwhile ions of same charge will be repelled. A so-called double layer of charges will be formed, consisting of a stationary and a diffuse phase, which stretches out for some distance from the silica wall or the particle surface. The surface between the phases is called the “slip surface” and the electric potential drop between the ionized surface area and the slip surface is defined as the zeta potential. This zeta potential will fall to zero, over a certain distance in the diffuse layer, known as the double layer length, δ . The zeta potential is related to δ and to the surface charge σ by the following equation [7,50]:

$$\zeta = \frac{\sigma \delta}{\varepsilon_0 \varepsilon_r} \quad (7)$$

By applying a voltage difference, the solvated ions in the diffuse layer will be attracted towards their electrode, dragging the solvent molecules with them [36]. Hence, to maintain the same electro-osmotic mobility a uniform zeta potential is needed in the capillary. In a packed capillary, where the zeta potential will be defined by the charged particles, a difference in inter particle porosity occurs at the wall, compared to the middle of the capillary. This non-uniformity of the EOF is worsened with non-homogeneously packed columns, whereby typically a lower packing density is observed close to the wall. This wall effect lowers exponentially with the distance to the wall, but creates a mismatch in the zeta potential and hence leads to a less uniform mobility and mobile phase velocity. Therefore, to implement kinetic plots, a uniform or as close as possible uniform bed is necessary.

In this contribution the KPL method is applied for the evaluation of the performance of packed columns in CEC with realistically retained solutes which contain both hydrophobic and polar functionalities. Comparison with HPLC is performed and its influence of temperature on the CEC performance is investigated. The approach is used to estimate the future potential of CEC in a clearer way.

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