



## Review

# A critical overview of non-aqueous capillary electrophoresis. Part II: Separation efficiency and analysis time



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## ABSTRACT

A survey of the literature on non-aqueous capillary zone electrophoresis leaves one with the impression of a prevailing notion that non-aqueous conditions are principally more favorable than conventional aqueous media. Specifically, the application of organic solvents in capillary zone electrophoresis (CZE) is believed to provide the general advantages of superior separation efficiency, higher applicable electric field strength, and shorter analysis time. These advantages, however, are often claimed without providing any experimental evidence, or based on rather uncritical comparisons of limited sets of arbitrarily selected separation results. Therefore, the performance characteristics of non-aqueous vs. aqueous CZE certainly deserve closer scrutiny. The primary intention of Part II of this review is to give a critical survey of the literature on non-aqueous capillary electrophoresis (NACE) that has emerged over the last five years. Emphasis is mainly placed on those studies that are concerned with the aspects of plate height, plate number, and the crucial mechanisms contributing to zone broadening, both in organic and aqueous conditions. To facilitate a deeper understanding, this treatment covers also the theoretical fundamentals of peak dispersion phenomena arising from wall adsorption; concentration overload (electromigration dispersion); longitudinal diffusion; and thermal gradients. Theoretically achievable plate numbers are discussed, both under limiting (at zero ionic strength) and application-relevant conditions (at finite ionic strength). In addition, the impact of the superimposed electroosmotic flow contributions to overall CZE performance is addressed, both for aqueous and non-aqueous media. It was concluded that for peak dispersion due to wall adsorption and due to concentration overload (electromigration dispersion, leading to peak triangulation) no general conjunction with the solvent can be deduced. This is in contrast to longitudinal diffusion: the plate height (and the plate number) obtainable under limiting conditions (at zero ionic strength) has the same ultimate value for all solvents. However, in background electrolytes with finite ionic strength, the maximum reachable plate number depends on the solvent, and in water it is higher than in the most commonly used organic solvents: methanol and acetonitrile. Thermal peak broadening is also larger in the organic solvents if compared to aqueous solutions under comparable conditions. However, its influence on the plate height is negligible under conditions established with commercial instrumentation. From the laws of electric and thermal conductance, it follows that no general conclusion can be drawn that with organic solvents higher field strength can be applied and shorter analysis time can be reached; the contrary is more evident: under comparable conditions aqueous solutions lead to more favorable results. This comprehensive analysis provides strong evidence that the broadly held notion of non-aqueous CZE being *principally superior* to aqueous CZE is a myth rather than a fact. However, several studies in which the employment of non-aqueous conditions has been instrumental to solve challenging analytical problems demonstrate that the intelligent use of non-aqueous CE has and will continue having its place in modern separation science.

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## 1. Introduction

Two main phenomena are fundamental for the separation of compounds in capillary zone electrophoresis (CZE): differential migration of the sample zones and the dispersion the zones underlie during their movement through the separation capillary. Differential migration of analytes is a combined function of the individual electrophoretic mobility of the sample constituents, and the electroosmotic mobility under the employed separation conditions. The electrophoretic mobilities of a given analyte depend on its size and charge in the corresponding medium; thus, especially for weak electrolytes, electrophoretic migration will be strongly influenced by the nature of the employed solvent (see e.g., Refs. [1–4]). With the velocity of the superimposing electroosmotic flow (EOF) contributing to the total migration velocity of the sample zones, the effect of the solvent on the electroosmotic increment also becomes a crucial component for separation selectivity. The work concerning the impact of the nature of the solvent on selectivity in non-aqueous capillary electrophoresis (NACE) has been discussed in Part I of this review [5], preferentially covering the studies that have been published over the last five years.

However, the quality of NACE separation, that is, the level of resolution that can be achieved between two zones migrating with different velocity, is ultimately governed by the extent of broadening these zones, which is experiencing during migration. Several processes are responsible for this zone dispersion, including thermal effects; analyte adsorption onto the capillary wall; mismatch between the mobilities of the analyte and the co-ions present in the background electrolyte (BGE) (leading to the so-called concentration overload or electromigration dispersion); longitudinal diffusion phenomena; and the parabolic velocity profile of the flow resulting from unbalanced fluid levels in the buffer reservoirs. Fundamental theories concerning these zone-broadening effects have been established for capillary electrophoresis (CE) in aqueous solutions, and can be readily applied, after proper modification, to non-aqueous systems. In Part II of this review, we will provide an overview of these adapted concepts, and demonstrate their value for the critical assessment of peak dispersion phenomena in NACE. As in Part I, this review will preferentially – but not exclusively – cover the relevant literature that has been published over the last five years.

Given the fact that within the CE community a rather optimistic view concerning the advantages of non-aqueous systems prevails, a critical and fact-based evaluation of merits and limitations emerging from the use of organic solvents appears to be indicated. Viewed from the analyte perspective, improved solubility and the higher separation selectivity are claimed as benefits (these aspects have been covered in detail in Part I of this review). Concerning overall separation performance of NACE, the following additional benefits are frequently claimed:

(i) Higher separation efficiency (plate numbers) can be obtained.

(ii) Generally in organic solvents, lower electric current and lower Joule heat allow higher voltage and result in shorter analysis time.

The following section of the review will attempt to dissect the myths from the facts concerning the advantages of the use of organic solvents for CE applications. This will be accomplished by identifying physico-chemical properties of the analyte and of the separation systems crucially contributing to band broadening, followed by a discussion of how these parameters are modified by given organic solvents. This approach will provide the approximations of the upper limiting separation efficiencies that can be realized in aqueous and non-aqueous solutions, respectively, and will also allow sound estimations of separation efficiencies experimentally obtainable under real-world conditions. These fundamental discussions will also cover the general aspects of applicable field strength and analysis time in context with the use of organic solvents in a general manner, apart from specific examples and conditions. In the best case, it allows to find the causes for the deviations of the experimental results from those theoretically expectable.

The organic solvents that have been most frequently employed in NACE application are methanol (MeOH), acetonitrile (ACN), and mixtures of both. Other solvents are formamide (FA), *N*-methylformamide (NMF), *N,N*-dimethylformamide (DMF), *N,N*-dimethylacetamide (DMA), propylene carbonate (PC), dimethylsulphoxide (DMSO), and nitromethane (NM). The impact of these solvents on solubility, mobility, and selectivity has been discussed in Part I of this review [5]; they are also topic of the current Part II.

## 2. Discussion

### 2.1. Separation Efficiency

To describe separation efficiency in CE, that is, the extent of zone broadening in a system, the same concept of the plate height,  $H$ , and the plate number,  $N$ , is applied in zone electrophoresis as in elution chromatography. The plate height is the factor of proportionality to which the spatial peak width, expressed by the variance,  $\sigma_x^2$ , is increasing with increasing migration distance,  $x$ . If it is measured after migration length,  $L$  (in CZE the distance between injector and detector), the plate height is expressed as  $H = \sigma_x^2/L$ . The plate number, crucial for separation, is  $N = L/H$ . As peaks are usually recorded in the time domain,  $N$  is calculated by the temporal variance  $\sigma_{t,i}^2$  of compound,  $i$ , and its migration time  $t_{m,i}$ :  $N = (t_{m,i}/\sigma_{t,i})^2$ ; if the width,  $w_{1/2}$ , at halve peak height rather than  $\sigma_{t,i}^2$  is considered, the plate number results as  $N = (t_{m,i}/w_{1/2})^2 \times 5.54$ .

Prior to a more detailed discussion about separation efficiency in CZE, it must be pointed out that some important differences between aqueous and organic solvent systems have to be taken into account. This shall be exemplified using NACE efficiency reported in recent literature. In one article [6], plate numbers of  $2.92 \times 10^4$ ,

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