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Review

High-sensitivity capillary and microchip electrophoresis using electrokinetic supercharging preconcentration Insight into the stacking mechanism via computer modeling

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ABSTRACT

This review discusses recent progress in the application of one of the most effective in-line preconcentration techniques used in electrophoresis in capillaries and microchips, electrokinetic supercharging (EKS). Conventionally considered as a transient isotachophoresis (tITP) step put into effect after the electrokinetic sample injection (EKI), EKS presumes that the electrolyte filled into the capillary (or microchip channel) comprises a co-ion acting as a leading ion to stack the injected analytes. Subsequently, to create the tITP state, one needs an additional injection of a suitable terminating ion. As a resulting increase in sensitivity strongly depends on the performance of both EKS stages, two theoretical sections are focused on hints for proper arrangement of EKI and tITP elaborated by means of computer simulation. In particular, factors affecting the injected amount of analytes, different modes of introducing the sample, suitable combinations of leading and terminating ions, and optimization of supporting electrolyte compositions are discussed with an objective to increase the enrichment factors. A comprehensive coverage of recent EKS applications in capillary and microchip electrophoresis, including metal ions, pharmaceuticals, peptides, DNA fragments, and proteins, demonstrates attainable sensitivity enhancements up to two orders of magnitude. This should make this method exportable to other analytes and facilitate its more widespread use to applications that require low limits of detection.

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1. Introduction

Over the past decades of successful development, CE has matured as an established separation technique in several application areas with recognized advantages of simplicity, separation efficiency, minor sample and solvent consumption, etc. Nevertheless, as a routine tool, CE is only applied in certain biotechnological and clinical fields, particularly, for analyzing serum proteins and disease markers. Outside of these irreplaceable quarters, analysts in many industrial and environmental laboratories continue to impede the uptake of CE due to the predominance of HPLC, as well as personal inexperience and suspicion of CE. The latter issue is mostly related to marginal limits of detection (LODs) attained using CE techniques. Low concentration sensitivity inevitably stems from minute sample volumes (at the nl level) and short optical pathlengths (typically 25–100 μm) available with commercial CE instrumentation.

In response to the sensitivity challenge, many efforts of CE developers have been focused on in-line preconcentration techniques [1–9]. Whilst realized through different principles, the majority of these are based on a stacking phenomenon in which analytes from a long diluted sample zone injected in the capillary accumulate into a much shorter zone (stack) and thus become concentrated prior to electrophoretic separation. Recently, Malá et al. [7] presented an explicit view on the basic principles underlying the sample stacking techniques while Breadmore [8] presented a clear-cut classification of various preconcentration modes involving stacking. One of these approaches, classified as a sequential stacking method, takes combined advantages of *electrokinetic sample injection (EKI)* and *transient isotachophoresis (tITP)* and has originally been introduced by Hirokawa et al. [10] under the name 'electrokinetic supercharging' (EKS). As EKS remains a relatively novel stacking method, its operational principles are still indistinctly understood. This reduces the resulting preconcentration effect and retards method's practical application. Such unwelcome situation underscored the necessity of the present review. Some basic discussions are given to emphasize the principle and influential parameters of EKI and tITP applied to CE and microchip electrophoresis (MCE) analysis. The stacking mechanism of EKS is examined and illustrated by computer simulations. A high-sensitivity potential of EKS and its applicability to complex samples are highlighted by an inclusive coverage of references, encompassing small ions, large molecules, and biopolymers.

2. Basic principle of sample stacking

Almost all stacking techniques are based on changing the electrophoretic velocity of sample components during their migration process, e.g. decreasing the electrophoretic velocity of sample components when they enter the capillary or the microchip separation channel. The most generic, electromigration stacking is attributed to electric field discontinuity throughout the separation medium. This forces the charged analytes to decrease the velocity and to become stacked at the boundary between a high electric field zone (sample) and a low electric field zone (separation electrolyte). The electric field-induced stacking mechanism is, for instance, the base of field-amplified sample stacking [11], field-amplified sample injection [12], and large-volume sample stacking techniques [13]. In addition, the requested reduction in effective mobility can be gained by changing the pH (dynamic pH junction [14]), formation of complexes or interaction with micelles (sweeping) [15], all supplementary effects generating selective stacking of analytes, as well as by physical approaches such as membrane filtration [16,17] or molecular sieving [18]. As a stacking method relying on different electric field distribution, EKS enables introduction of a large amount of diluted analytes but without deteriorating their separation, a unique process which will be detailed below.

3. Factors controlling electrokinetic injection

In EKI, the sample introduction process is driven by a combined effect of electrophoretic mobility and electroosmotic flow (EOF) and has an intrinsically stacking character, depending on the applied voltage and ionic strength of the sample solution and the background electrolyte (BGE).

3.1. Mobility bias

Jorgenson and Lukacs were the first to point out that the EKI discriminates between analytes on the basis of their mobility [19]. Chien and Burgi [20] defined the amount of analyte ions injected (N_i) as

$$N_i(t_{\mathrm{inj}}) = \pi r^2 c_i(\mu_{\mathrm{eof}} + \gamma\mu_i)E_{\mathrm{inj}}t_{\mathrm{inj}} \quad (1)$$

where t_{inj}, c_i, μ_i, and μ_{eof} represent the injection time, the concentration of a given analyte in the sample, the mobility of the analyte, and the mobility of EOF, respectively. The symbol γ stands for the potential gradient ratio (the ratio of the resistivity of the sample to the BGE), and E_{inj} is field strength applied across the capillary length for sample introduction. As can be seen from Eq. (1), the amount injected by EKI depends on the mobility of the analyte, giving rise to mobility bias within one sample or between samples [21–23].

Since EKI is an ion-transport phenomenon, it could be described taking into account electric charge variations. The magnitude of N_i should linearly depend on the applied charge and the transference number of the analyte (T_i) in the sample. Fig. 1 displays the relationships between the injected amount and the time of injection and the applied charge obtained by computer simulation. The model sample consisted of five cations with the assigned mobility of 50×10^{-5} ($m50^+$), 40×10^{-5} ($m40^+$), 30×10^{-5} ($m30^+$), 20×10^{-5} ($m20^+$), and 10×10^{-5} ($m10^+$) $\mathrm{cm^2\,V^{-1}\,s^{-1}}$, respectively, and with the same concentration of 0.01 mM. Note that a zone of leading electrolyte (LE) was introduced before EKI (see Section 4). Two significant occurrences follow from the simulation experiment: (i) the ratio of N_i among the analytes is proportional to the mobility of the analyte in the mixture and (ii) a good linear relationship exists between the injected amount and applied charge (the product of μ_i and c_i) when the EOF is not assumed (Fig. 1b); the latter factor will be in detail discussed in the following section.

3.2. Effect of sample conductivity and electroosmotic flow

Stacking by means of EKI is based on that the conductivity of the sample is (much) lower than that of the BGE, which leads to field discontinuity along the migration path. Therefore, the approach to

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