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Quantitative and qualitative precision improvements by effective mobility-scale data transformation in capillary electrophoresis analysis

By transforming the time-based *x*-axis of electropherograms in capillary zone electrophoresis (CZE) into the corresponding effective mobility-scale, we propose a simple and robust data representation for a better qualitative and quantitative capillary electrophoresis (CE) analysis. The time scale of the raw electrophoretic data (detection signal *versus* time) is transformed into an effective electrophoretic mobility scale (μ_{eff}-scale) with account of the electroosmotic flow (EOF) peak or of an internal standard of known effective mobility. With the new scaling (detection signals *versus* effective mobility), the obtained electropherograms are more representative of the velocity-based electrophoretic separation and the comparison of complete electropherograms is directly possible. This is of importance when tracking peaks in real samples where alteration in EOF stability can occur or when comparing electrophoretic runs from different experimental setups (independence in column length and voltage). Beside the qualitative possibilities, a quantitative improvement is achieved in the μ_{eff}-scale with significant better peak area reproducibility and equal to more precision in quantitative analysis than with the primary time-scale integration.*

Keywords: Precision / Capillary zone electrophoresis / Data processing / Mobility-scaling / Qualitative and quantitative analysis EL 4188

1 Introduction

Capillary electrophoresis (CE) became an important and efficient separation tool in biosciences and environmental chemistry over the last decade [1]. With the first commercial instruments at the end of the 80ies, a relative standard deviation (RSD) in peak areas and heights higher than 5% was found, which is too poor for quantitative analysis. The instrumental developments lowered the RSD to around 1–2% mid of the 90ies, allowing now quantitation with all instruments. With the on-coming multiple injection mode, a day-to-day precision in the 0.1% range is even to be expected [2]. Besides instrumental improvements in the control/stabilization of the power supply and temperature, in the detection and the injection devices, the quality of the separation capillaries could also be improved. Meanwhile more and more CE methods are validated for pharmaceutical quality control [3], environmental analysis [4], or even systematic toxicological analysis [5]. The separation mechanisms themselves are also more and more understood, modeled, simulated and thus controlled. A "CE-mode-thinking" as called by Whatley [6] is, however, the prerequisite to handle CE problems and reach good reproducibility.

The low reproducibility in migration time and peak integration parameters is often related to little changes in the electroosmotic flow (EOF) due to uncontrollable alterations of the capillary surface, leading to some not always understandable migration time shifts especially when analyzing real samples (matrix effects). A first step in achieving precision is the proper operating, calibrating and equilibrating of the separation system leading to a stable EOF and reproducible migration times. This can be reached with different experimental setups as for example adequate rinse steps [7, 8] or voltage preconditioning techniques [9]. Some optimizations of the precision were reviewed recently and showed both instrumental and methodological improvement possibilities [10].

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Abbreviation: **μ-scale**, electrophoretic mobility scale

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The CE-softwares for the control and the data processing of the measured signals with CE are mainly derived from the already existing programs of classical chromatography techniques (mainly high-performance liquid chromatography, HPLC) and allow essentially the description of the signal variation as a function of time (it might be called chromatography-mode-thinking). Electrophoretic separations, however, are not based on the same separation processes as in chromatography and the time-based plots are not representative of the fundamental quantity that is the velocity of the sample per unit of field strength of the sample (effective mobility, inversely proportional to time – $1/t$). Attempts to better represent these parameters in the electropherograms, leading to significant qualitative reproducibility enhancements, were proposed by other authors in plotting the signals *versus* the "quantity of electric charge, [11], the 1/time domain [12], the effective mobility [13–15], or using migration indices [16] and migration time ratios [17]. The use of the effective mobility of a analyte combined with a UV-visible spectral library as obtained by diode array detectors was proposed more recently as a decision tool for an accurate peak assignment [18]. These transformations increase significantly the reproducibility in the calculated parameters but allowed only qualitative improvements as useful tools for peak identification. No information on the quantitative consequences of such transformations have been reported in the literature up to now. This article is intended (i) to confirm the usefulness of the transformation of the total time *x*-scale of electrophoretic data into the corresponding effective mobility scale (μ-scale) for a better qualitative peak tracking of single components from complex matrices and (ii) to examine the possibilities of quantification by peak integration in the μ-scale.

2 Materials and methods

2.1 Instrumentation

The instrumentation consisted of a Beckman P/ACE 2050 (UV-filter used at 230 nm for the pesticides and 254 nm for phenolic acids), and a P/ACE 5510 (equipped with diode array detection DAD) with the corresponding Beckman system software (Palo Alto, CA, USA).

2.2 Separation

Uncoated fused-silica CZE columns (75 μm ID, 375 μm OD, 50 cm length to the detector, total length 57 cm, and 30 cm length to the detector, total length 37 cm, respectively) were obtained from Laser 2000 (Weßling, Germany). CZE conditions: 50 mM acetate buffer (pH 4.5) as described elsewhere for the separation of the three pesticides [19], 25 mM carbonate buffer (pH 9.3) for the separation of the phenolic acids; temperature, 30°C; voltage, 20–25 kV; hydrodynamic injection, 5–10 s. The capillary was washed with 0.1 M NaOH for 2 min before and after each run.

2.3 Chemicals

Fenoprop, mecoprop and dichlorprop were obtained from Riedel-de-Haen (pestanal grade; Munich, Germany). Benzoic-, *p*-hydroxy- and vanillic acid, as well as the chemicals needed for buffer preparation were obtained from Sigma/Aldrich (Deisenhofen, Germany).

2.4 Data processing

The raw electrophoretic data were treated with the use of the self-designed program "GelTreat" developed by Dr. Perminova and A. Kudryavtsev from the Department of Chemistry, Lomonosov Moscow State University, Russia. The program is described in [20] and was initially designed for the treatment of size-exclusion chromatograms of humic substances. To make it applicable for the treatment of electropherograms in mobility scale, the program was modified by the designer by addition of a block of the transformation of *x*-axis of the initial electropherogram to allow its conversion from the time-scale into the μ-scale. The transformation into the μ-scale is certainly possible with classical data processing programs but this self-designed program allowed the complete analysis of a data set much more rapidly.

2.5 Theoretical background

2.5.1 Mobility scale transformation

It is essential to remind some basic rules of CZE [21] that are at the origin of the proposed *x*-scale transformation. Each molecule has a specific effective mobility as a function of its own physicochemical characteristics (charge, size) with a given separation buffer (pH and ionic strength governing its charge and hydrodynamic radius). The measured electrophoretic mobility μ_{mes} (cm^2/Vs or $cm^2/Vmin$) is calculated from the measured electrophoretic velocity v_e (cm/s or cm/min) and the applied electric field strength E (V/cm), taking account of the migration time (t_{mes}), length of the capillary to the detector (L_d), the total length of the capillary (L_t), and the applied voltage (V):

$$\mu_{mes} = \frac{v_e}{E} = \frac{L_d L_t}{t_m V} \quad (1)$$

The measured migration time (t_m) and the corresponding measured mobility (μ_{mes}) do not reflect the velocity (directly correlated to the effective electrophoretic mobility

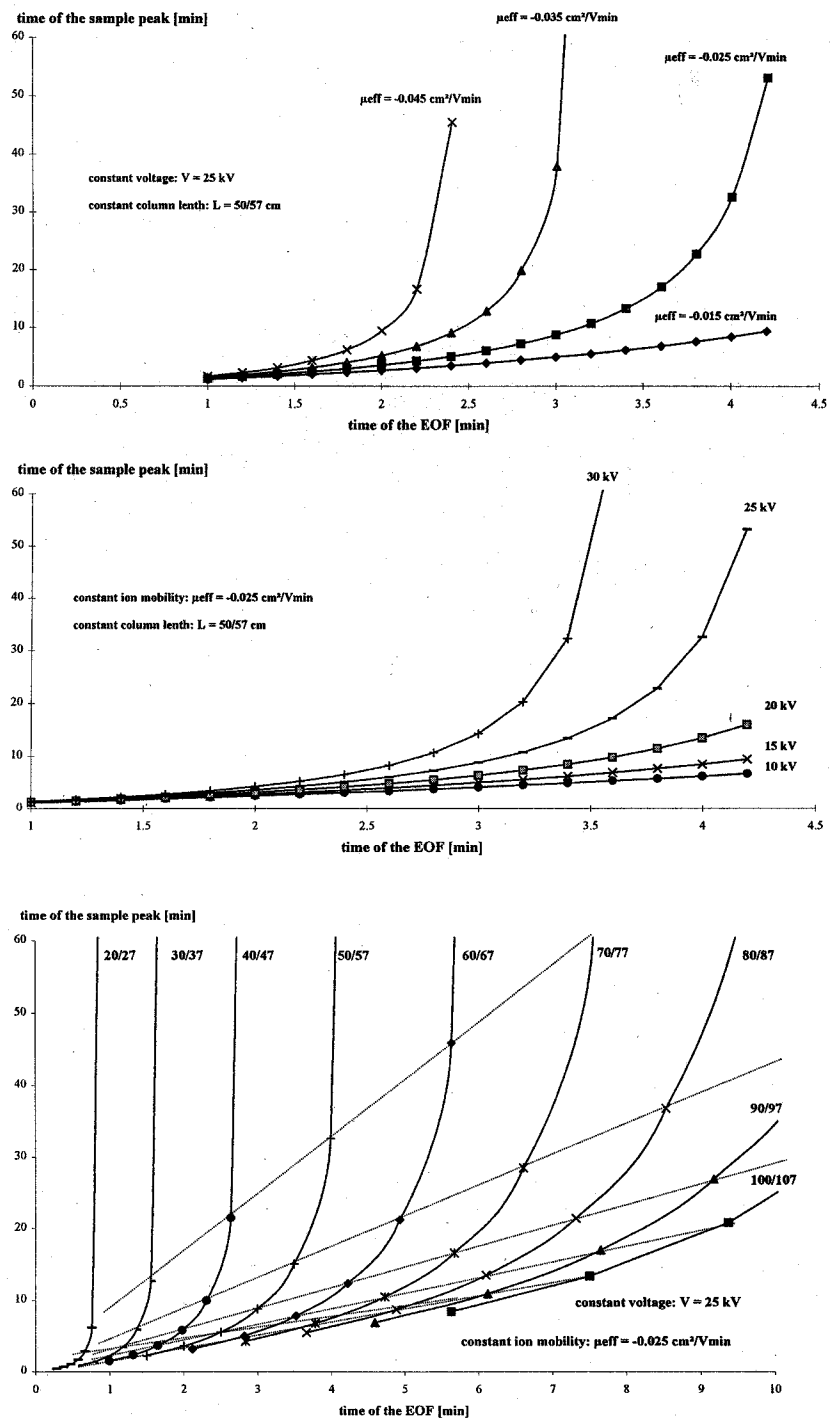

Figure 1. Theoretical variations of the observed migration time of a component as a function of the variation in the migration time of the EOF. (a) Influence of the effective mobility of the analyzed analyte, (b) influence of the voltage, (c) influence of the column length (the linear lines correspond to the iso-μ_{eof} lines, *i.e.*, changes of t_{eof} by change of the column length).

μ_{eff}) of the analytes in the separation system because it is also dependent on the EOF – acting as pump for the buffer to the cathode. The effective mobility can thus be regarded as a Vcm^{-1}-normalized velocity of the molecules in the capillary obtained by changing the reference system from the observer (time measurement of signals through the detection device) to the buffer system itself; this absolute value becomes independent of the used lengths, voltages, and even buffer velocity fluctuations (EOF changes). The effective electrophoretic mobility (μ_{eff}) of the analytes is calculated by subtracting the EOF (μ_{eof}) from the measured electrophoretic mobility (μ_{mes}) – EOF-correction – and is used as an absolute electrophoretic value. Its value is negative in sign for anions and positive for cations:

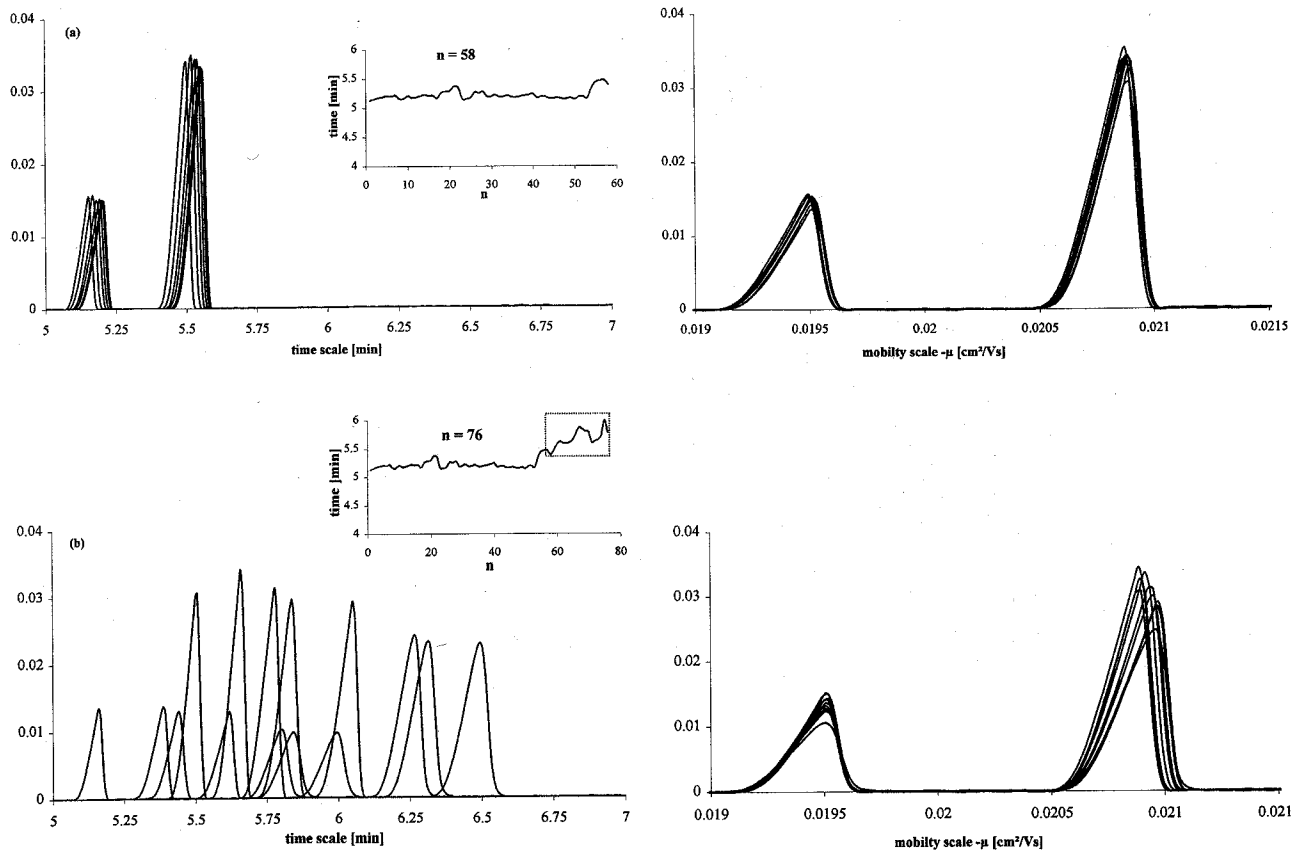

Figure 2. Selected electropherograms and corresponding μ-scale of *p*-hydroxybenzoic acid (left peak) and vanillic acid (right peak) in (a) the $n = 58$ reproducibility study and (b) $n = 76$ study with induced migration time shifts. The variation of the migration time of *p*-hydroxybenzoic acid in these two series is also shown for $n = 58$ and $n = 76$ (58 + 18).

$$\mu_{eff} = \mu_{mes} - \mu_{EOF} \tag{2}$$

$$\mu_{eff} = \frac{L_d L_t (t_{eof} - t_m)}{V t_m t_{eof}} \tag{3}$$

During the measurements, the detection signals (from UV/Vis, LIF, *etc.*) are plotted against time-signal = $f(t_m)$. Transforming the data into the μ-scale does not result in any loss in information because of the bit-to-bit correspondence like the transformation into the $1/t$ domain or in infrared spectroscopy from wavelength to frequency terms [12]. The input parameters for the transformation in the μ_{eff}-scale are only L_d, L_t, V, and t_{eof} (the EOF-peak is determined manually after addition of mesithyl oxide) according to Eq. (3), signal = $f(\mu_{eff})$.

If an internal standard with known (or measurable) mobility μ_{int} (time t_{int}) is used, the transformation is similar by calculating first t_{eof} from Eq. (3) and substituting the value of t_{eof} to Eq. (3) to obtain the signal as a function of μ_{eff}. The software was written for these two alternatives. Thus one obtains:

$$\mu_{eff} = \mu_{int} + \frac{L_d L_t (t_m - t_{int})}{V t_m t_{int}} \tag{4}$$

2.5.2 Some origins in migration time fluctuations

When assuming only little changes in the buffer viscosity (a parameter that is nearly impossible to be measured systematically in routine laboratory practice), *i.e.*, when operating at constant temperature, Eqs. (1) and (3) govern the changes of the migration time (t_m) of a component with the EOF (t_{eof}) as a function of the column lengths (L_d and L_t), the applied voltage (V), and the effective mobility (μ_{eff}) of the analyzed molecule (μ_{eff} keeps a constant value in the same separation buffer). We illustrated in Fig. 1a the relation between these key parameters by plotting the migration time (t_m) *versus* the time of the EOF flow (t_{eof}). The four chosen μ_{eff} may correspond to four

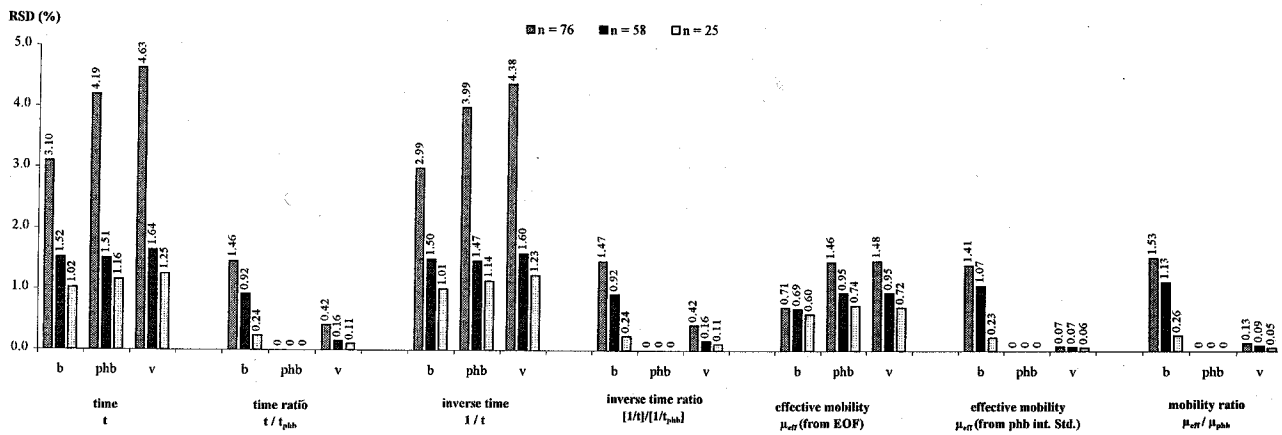

Figure 3. Relative standard deviations (RSD%) in migration time (t), migration time ratio (t/t_{phb}, with t_{phb}, the time of phb taken as internal standard), inverse migration time ($1/t$), relative to *p*-hydroxybenzoic acid inversed migration time ($[1/t]/[1/t_{phb}]$), effective mobility (μ) calculated from neutral-peak as EOF-marker (Eq. 3), the corresponding effective mobility ratio (μ/μ_{phb}), and the effective mobility calculated taking *p*-hydroxybenzoic acid as internal standard from time-scaled electropherograms. b, benzoic acid; phb, *p*-hydroxybenzoic acid; v, vanillic acid.

components bearing 1, 2, 3, and 4 charges, respectively (*e.g.*, benzoic, phthalic, trimellitic, and pyromellitic acid in alkaline pH). Already small fluctuations in the EOF from one measurement to another can have great effects on the migration time of the component. For example, at 25 kV and with a 60/67 cm column, the change in EOF from 2.2 to 2.6 min would induce a shift in the migration time of a very charged molecule from 13.4 min to over 60 min. Molecules with lower mobility, however, would not be affected so much (Fig. 1a). This effect is even increased for higher applied voltages (Fig. 1b) and lower column lengths (Fig. 1c). The dotted line in Fig. 1c corresponds to the linear change in the time of the EOF by changing the column length.

Small variations in the EOF affecting the migration time of a component (and thus the reproducibility of the observed electropherogram) may occur when analyzing samples from real matrices [22] or trying to follow the variation in mobility of the sample by addition of some ligands in the separation buffer within affinity CE studies [23, 24]. However, in identical separation buffer conditions, the effective mobility of a component is per definition constant and independent of any changes in EOF. As a response to this fact, we proposed a representation of the primary data in the μ-scale [13–15]. The plots of the measured signal in the $1/t$ domain (possible in an online modus) has already been proposed by other authors as a useful way of representation of the electropherograms [12]. But even though the difference between two peaks becomes a linear function of their difference in mobility in the $1/t$ domain, variations may occur when the EOF is not stable within a measurement series and different separation conditions (column length, voltage) can not be compared directly.

3 Results and discussion

The calculation of the effective mobility of single peaks – as present now in most of the CE software packages – becomes a more and more accepted tool for a precise identification of peaks especially when measuring real samples where shifts in times result from EOF drifts. The μ-scale representation is only the extension of this CE-mode-thinking to the whole data of an electropherogram allowing thus a direct analysis of the total data sets. Qualitative and quantitative implications of such a transformation are shown here.

3.1 Qualitative implications of scale changes

3.1.2 Effects on repeatability and reproducibility

To study the repeatability and reproducibility of the data, a series of 76 measurements of a standard mixture of benzoic (b), *p*-hydroxybenzoic (phb) and vanillic (v) acids has been carried out over a period of two days. Within this series, the first 25 measurements corresponded to the same batch of samples analyzed successively in an automatic mode, *i.e.*, under experimental conditions controlled

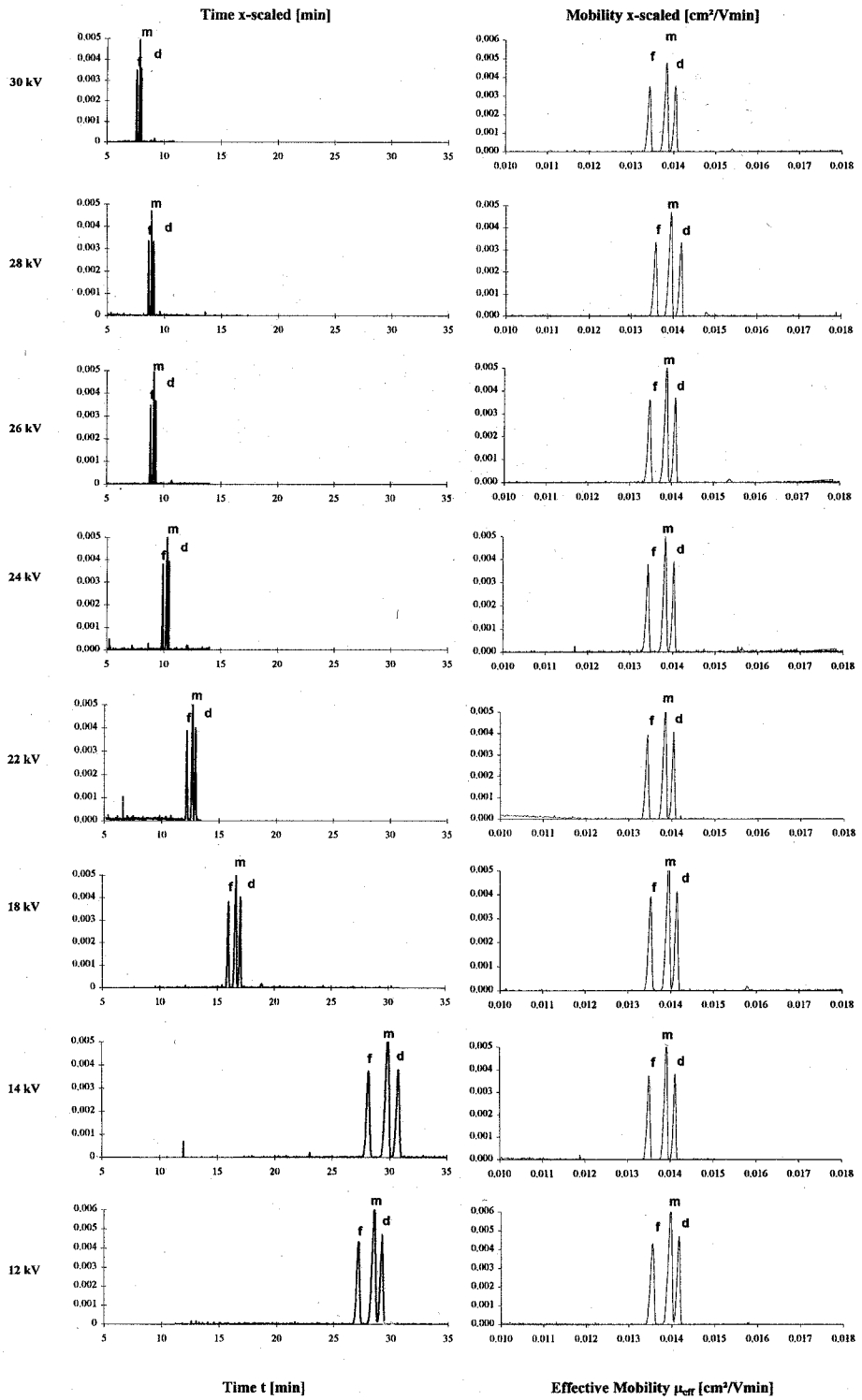

Figure 4. Electropherograms of the separation of three phenoxy acids (f, fenoprop; m, mecoprop; d, dichlorprop) with varying separation voltage from 12 to 30 kV in time-scale and in the transformed μ-scale (transformation from EOF-peak).

as strictly as possible. The subseries of 58 measurements was carried out using the different batches of samples. Finally, 18 further separations were conducted with the same outlet vials to induce slight pH changes and a systematic shift in the EOF – and thus high variations migration times (up to 1 min). *p*-Hydroxybenzoic acid was taken as an internal standard for the data μ-scale transformation. Therefore, the entire set of $n = 76$ and its subset of $n = 58$ were used to evaluate the parameters of reproducibility, the subset of $n = 25$ those of repeatability.

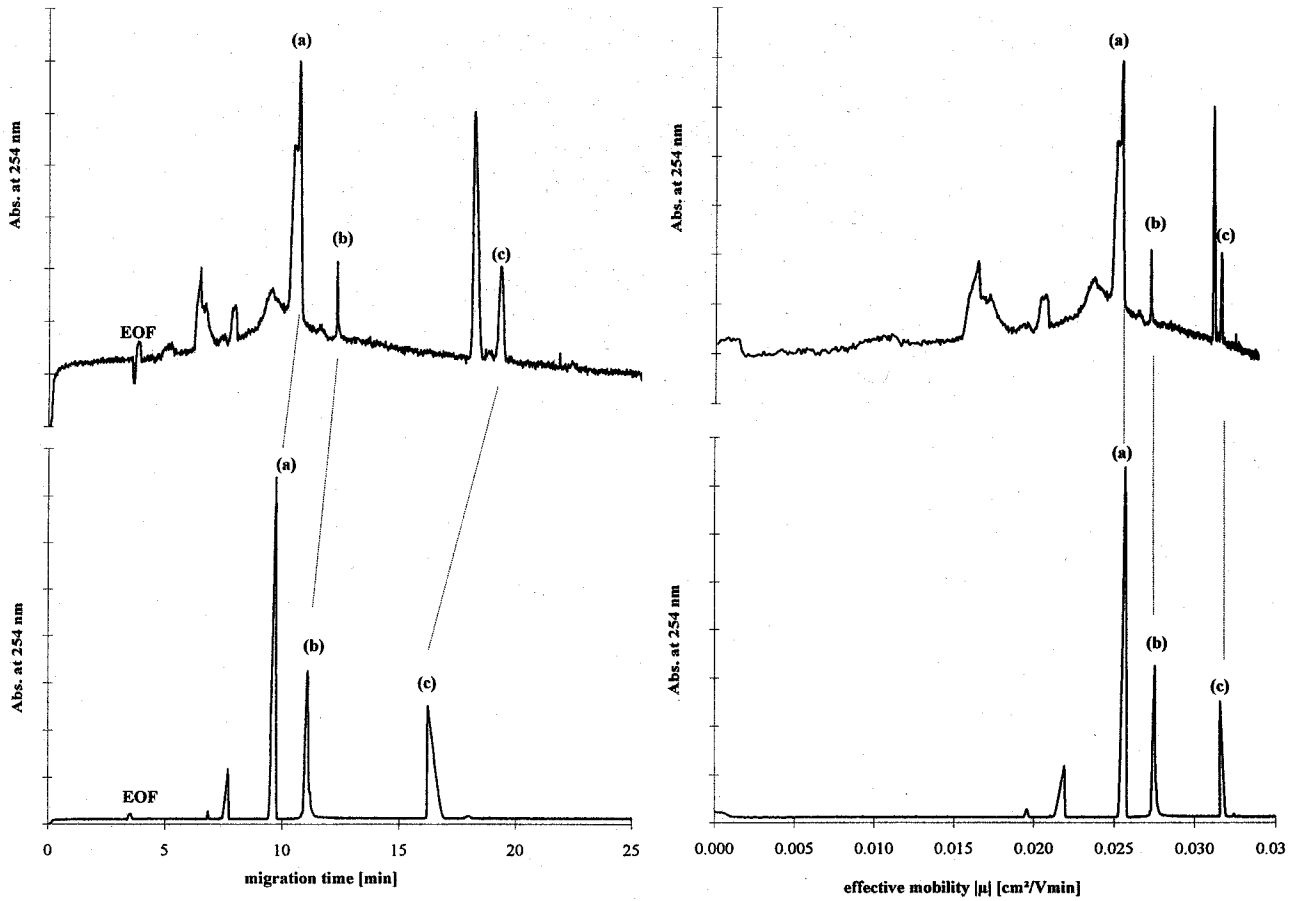

Figure 5. CZE-electropherograms of lake water fulvic acids after their inoculation with microorganisms isolated from groundwater; the electropherograms of degraded fulvic acids are shown in time and mobility scales with the corresponding phenol standards in the same experimental conditions (a, benzoic acid; b, protocatechuic acid; c, phthalic acid).

Selected electropherograms and their corresponding μ-scale electropherograms are given in Fig. 2a and show the high stability in the mobility scale. The shifts are illustrated in Fig. 2b as well as the corresponding more stable electropherograms in μ-scale. For the three data sets (n = 25, n = 58, and n = 76) we compared the RSD in migration time (t), migration time ratio (t/t_{phb}, with t_{phb} the time of phb taken as internal standard), inverse migration time ($1/t$), relative to phb inverse migration time ($[1/t]/[1/t_{phb}]$) with the RSD in mobility of the same analytes. In the latter case, we distinguished the effective mobility (μ) calculated from neutral-peak as EOF-marker (Eq. 3), the corresponding effective mobility ratio (μ/μ_{phb}) and the effective mobility calculated taking phb as internal standard (Eq. 4 setting $\mu_{int} = \mu_{phb} = -0.020$ cm^2/Vmin for all mobility calculations). These results are given in Fig. 3. With all data sets, an improvement in the RSD is obtained as expected by using the migration time ratios, the $1/t$ domain or the effective mobility. Within the repeatability study (n = 25) where the experimental condition could be held as closely as possible, the effective mobilities calculated with phb as internal standard showed an RSD lower than 0.25%. The two other data sets, measured over a longer period of time, include variations due to the change of vials in the instrument (n = 58) as well as little buffer condition changes (n = 76). In both cases, the calculated mobility ratios and effective mobilities stayed in a good RSD range under 1.5%.

3.1.3 Implications when changing experimental conditions

Because of the possible changes in migration times of the analytes with little changes in the EOF (as seen in Fig. 1 and Fig. 3), the transformation of the primary data in mobility x-scale (μ-scale) allows a way of data representation with a very good reproducibility (their effective mobility is theoretically independent of the EOF changes). This can be very valuable when trying to identify components from complex mixtures (when migration time shifts are caused

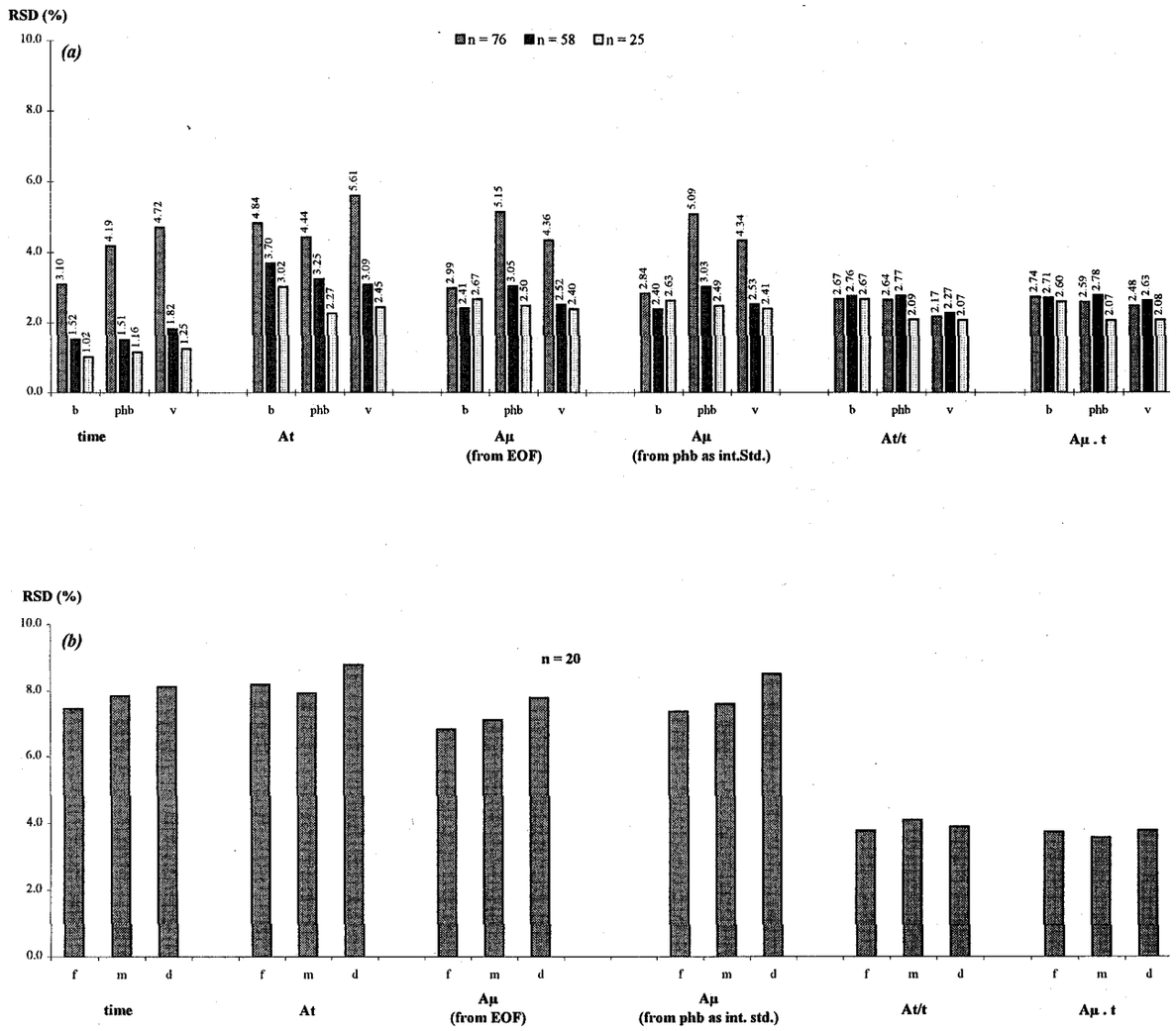

Figure 6. Relative standard deviations (RSD%) in migration time, area from time-scaled electropherograms, area from μ-scaled electropherograms, and corresponding corrected areas of m, mecoprop; f, fenoprop; d, dichlorprop in (a) the $n = 76$, $n = 58$, and $n = 25$ measurement series and (b) long-time reproducibility study (three weeks). *p*-Hydroxybenzoic acid was used as internal standard).

by little EOF changes). A first example showing the stability of this new representation is given in Fig. 4 with the separation of three phenoxy acid pesticides (f, fenoprop; m, mecoprop; d, dichlorprop) getting migration times from 7 to 27 min at different voltages (30 and 12 kV, respectively). Changing the voltage has also a direct influence on the peak width (peak heights remain in the same range). The variation in peak area between the runs at 12 and 30 kV (with identical buffer and column conditions) is around 900% from the time-scaled electropherograms while it is below 10% for the μ-scaled electropherograms showing a higher stability in mobility scale as determined in Section 3.2. The change in the speed of the analytes in the capillary and of the EOF due to the voltage, induced the changes in migration time of the components in the time-scale electropherograms according to the theoretical curves in Fig. 1b. The normalization in μ-scale of these primary data gives the electropherograms for each separation voltage, respectively. Because the intrinsic mobilities of the phenoxy acids are unchanged and independent of the separation conditions, the obtained μ-scale electropherograms for the same voltage-series data look identi-

Table 1. Relative standard deviations (RSD%, $n = 3$) in areas A_t. A_μ and corrected areas A_t/t and $A_\mu t$ of fenoprop, mecoprop and dichlorprop at different voltages, concentrations, and injection times.

Injection time	2 s				5 s				10 s				20 s			
	A_t	A_μ	A_t/t	$A_\mu t$	A_t	A_μ	A_t/t	$A_\mu t$	A_t	A_μ	A_t/t	$A_\mu t$	A_t	A_μ	A_t/t	$A_\mu t$
Fenoprop	3.34	3.07	4.16	2.17	6.65	2.79	5.59	3.61	1.82	0.64	0.71	0.67	4.01	0.29	2.06	2.08
Mecoprop	4.43	3.01	5.06	2.37	7.42	4.45	6.16	5.24	1.97	1.16	0.52	0.50	5.50	1.73	3.38	2.90
Dichlorprop	3.35	2.49	4.10	1.51	7.58	3.97	6.10	4.99	2.62	0.72	1.02	0.96	5.95	1.60	3.51	3.59

Concentration	1 ppm				2 ppm				5 ppm				10 ppm			
Fenoprop	0.62	1.34	1.39	1.37	1.80	2.43	0.40	0.33	1.75	2.12	2.63	1.54	3.63	2.75	3.13	3.24
Mecoprop	1.92	0.43	1.21	1.34	2.52	1.75	0.38	0.55	0.74	0.86	1.55	0.53	4.07	1.41	3.86	1.39
Dichlorprop	2.72	1.67	1.65	0.32	3.09	2.11	0.67	0.43	2.81	0.45	3.74	1.37	4.27	3.64	3.97	3.91

Voltage	12 kV ($n = 2$)				18 kV				22 kV				24 kV			
Fenoprop	3.95	2.60	3.57	2.11	5.2	1.08	2.2	1.80	2.4	1.14	1.3	1.87	4.1	1.48	2.9	0.67
Mecoprop	4.12	3.14	4.10	2.24	5.3	0.79	2.4	1.81	2.7	0.88	1.5	2.23	4.5	1.66	2.8	0.24
Dichlorprop	4.73	3.17	4.23	2.59	5.6	1.08	2.2	1.96	2.7	0.79	1.4	1.79	4.3	0.90	2.3	0.47

Voltage	26 kV				28 kV				30 kV			
Fenoprop	4.38	3.42	1.8	1.5	4.28	1.6	1.01	2.18	9.0	2.82	2.9	4.4
Mecoprop	4.90	3.41	2.2	2.0	3.61	2.3	0.65	2.89	9.5	3.80	3.5	4.3
Dichlorprop	5.61	3.93	3.1	2.6	3.88	1.7	0.54	2.18	10.5	2.23	4.2	2.8

cal. The effective mobility (expressed in $cm^2/Vmin$ or cm^2/Vs) and its distribution within the capillary remains unchanged independently of the applied voltage and the capillary length.

3.1.4 Implications for unknown peak identification

Because of the sensible changes of migration times of the analytes with little changes in the EOF (as seen in Fig. 1), the transformation of the primary data in mobility *x*-scale (μ-scale) is very valuable when trying to identify components in complex mixtures. This is illustrated with the example in Fig. 5 where we tried to identify benzoic acid (a), protocatechoic acid (b), and phthalic acid (c) from a microbial degraded fulvic acid (HS). The results clearly showed significant alterations of the original humic structure of the studied lake HS under the influence of groundwater microorganisms [25]. The separation was done in a 25 mM carbonate buffer, pH 9.3, at 20 kV; both electropherograms on the left side are time-scaled. The EOF varied slightly (6%) from the upper to the lower electropherogram (from 3.63 to 3.41 min). The induced peak shifting makes the comparison of two electropherograms in time-scale and the peak identification very difficult. On the right side the representation of the same separation is done in the μ-scale; the peak correspondence is made directly very easily and could be verified by measuring at a different pH (pH 5.3 [25]), by spiking and by comparing the UV-spectra.

3.2 Quantitative implications of scale changes

In a first set of experiments, the previous data sets ($n = 25$, $n = 38$, $n = 76$) were subjected to peak integration in time- and mobility-scale. A positive correlation (in our example with R from 0.82 to 0.93, $n = 76$) between the area measured in time-scale (A_t) and the migration times (t) led to the definition (as in chromatography) of the time-corrected area (A_t/t) taken as quantification parameter in all CE experiments. When integrating in mobility scale, the peak areas (A_μ) are found to be negatively correlated to the migration time (in our example with R from –0.62 to –0.86); a time-corrected mobility-scale area can thus be defined as well as $A_\mu t$. The effective mobility corrected time-scale area was already presented by different companies in their software the last years as a possible correction to improve precision and reproducibility in CE [26, 27].

Figure 6 gives the relative standard deviations (RSD%) of the different parameters (t, A_t, $A\mu$ calculated from the EOF peak, A_μ calculated from the internal standard peak, A_t/t, and $A_{mu}t$) for benzoic acid, *p*-hydroxybenzoic acid, and vanillic acid in four different data sets. (i) Figure 6a includes the 76 consecutive measurements (over a continuous period of two days). The high variation in migration time (3–5%, electropherograms in Fig. 2b) induced variations in A_t from 5–6%. The RSD of the areas calculated from the μ-scale electropherograms (A_μ) are in the same range (3–5%) regardless of whether the transformation was done using the EOF (EOF marker mesithyl oxide) or *p*-hydroxybenzoic acid taken as internal standard (identical mobility taken for the 76 transformations). The corrected area (A_t/t and $A_\mu t$) are not significantly different and show the best RSD% for quantification (2–3%). (ii) The first consecutive 58 measurements shown in Fig. 5a have a good reproducibility in time (<2%). The area from μ-scale has a lower RSD% than A_t and the time corrections (A_t/t) do not bring any supplemental improvement in RSD. (iii) Within the repeatability data set ($n = 25$) there is only a slight improvement of the RSD% as measured with A_t/t and $A_\mu t$ as compared to the reproducibility data sets. (iv) Figure 6b show measurements with fenoprop, mecoprop, and dichlorprop in different experimental conditions over a period of three weeks with different column charges and daily fresh-made buffer systems. The variations in the uncorrected parameters are very high (>7%) and the time correction of the area is necessary to improve the reproducibility of the quantitative parameters (time corrected areas are lowered to 4% with both the time as with the μ-scale electropherograms). This shows in each of these cases that the integration of peaks as A_t/t or as $A_\mu t$ give similar RSD% values and, thus, that the effective mobility scale can not only be used for qualitative improvement in data analysis, but also for quantitative analysis.

In a second set of experiments, we tried to verify the possible integration in the μ-scale for quantification with the three phenoxy acid herbicides measured in triplicates at different voltages, sample concentrations, and injection times (voltage data from electropherograms in Fig. 4). The corresponding RSD% ($n = 3$) obtained are presented in Table 1. To test the statistical significance of the observed trend, the analysis of variance has been performed [28, 29]. At first, all data variances were divided into four groups corresponding to time-scaled (A_t), μ-scaled area (A_μ) data as well as the respectively corrected area data (A_t/t and $A_\mu t$), and the homogeneity of variances within each group was tested using the Cochran-test. The variances in all groups were found to be homogeneous at a significance level of $\alpha = 0.05$. Then the average variances for each group were calculated and compared using the one-sided Fisher-test. The experimental variance ratio obtained between A_t and A_μ (4.23), A_t/t (2.42) and $A_\mu t$ (3.90) are higher than Fisher's coefficient for $\alpha = 0.05$ with corresponding numbers of degress of freedom of the variances (90, 90) equal to 1.42. The variance ratio between A_t/t and $A_\mu t$ is 1.61. So, the results obtained in time- and μ-scales differ significantly by their precision, showing an expressive improvement in quantitation when performing the mobility-scale transformation of the raw electrophoretic data.

4 Concluding remarks

For possible routine analysis, CE techniques need to give the same qualitative and quantitative results from run-to-run and day-to-day measurements. The modern technology allows to reach these goals in terms of instrumentation. However, within electrophoretic separations, where the migration times of an analyte is directly related to the EOF, the "chromatographic-mode-thinking" and the way of data processing need to be adapted. Representing the electropherograms in the μ-scale brings both qualitative and quantitative advantages. The conversion of the primary time-scaled data in mobility scale (μ-scale) leads to a better interpretation of the obtained electropherograms in terms of separation processes, and the direct implications enable a better direct comparison of electropherograms and an easier "peak tracking" when trying to identify single components from complex matrices, especially when UV-visible signatures of the components are also available. The peak integration showed an equal to significant improvement of the precision when done in μ-scale over a wide sample concentration and voltage range as compared to the time-corrected time-scale areas. The same data treatment can be applied when comparing measurements done with columns of different lengths or upscaling methods from one instrument to the other. Furthermore, this representation was proven to be necessary when describing the distribution in effective mobility of polydisperse samples like charged synthetic polymers or natural humic substances [15]. The transformation is also applicable to other CE techniques were changes in EOF can alter the stability of migration times, *i.e.*, capillary gel electrophoresis (CGE), micellar capillary electrophoresis (MEKC), and affinity capillary electrophoresis (ACE). It is certainly unusual for the chromatography-mode-thinkers to make the transformation from the time-scale into μ-scale but probably "trivial" for the CE-mode-thinkers who are used to induce relative differences in the velocity of the molecules they wants to separate. Fact is that an electrophoretic-based data processing CE-software is needed to be able to handle directly the electrophoretic data with the CE-software.

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