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Efficient computerized data acquisition and evaluation for capillary isotachophoresis in quiescent and flowing solution with single detectors placed towards the capillary end

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ABSTRACT

The use of a commercial, inexpensive, computerized data acquisition system and different data evaluation schemes for isotachophoresis in capillaries is reported. Data acquisition features two channels, automatic range switching and a unique dynamic sampling rate between 0.0125 and 100 Hz which provides compressed data files and smoother signals compared with sampling at a constant rate. Software termed UNIclip was written for data evaluation based on zone length measurements of conductivity and/or UV absorption signals and the chromatographic integration software was employed for data evaluation based on peak areas of baseline-resolved UV absorption peaks. In a configuration with minimized electroosmosis, high-quality data with linear calibration graphs were obtained. In the presence of electroosmosis, and also electroosmosis combined with a vacuum generated co-flow, in principle UV absorbance data with excellent linear calibration and high reproducibility could be obtained. However, the occurrence of outliers and the detection of uneven zone plateaux made accurate quantification difficult using an automated instrument with a single detector placed towards the capillary end.

INTRODUCTION

In capillary isotachophoresis (CITP), computerized data acquisition and evaluation are prerequisites for automation [1–3], in addition to a more convenient and accurate approach for quantification compared with the customary use of strip-chart recorders and manual data handling [4,5]. During the past decade, computer-based data acquisition systems for laboratory-made [6,7] and commercial [2,3] instruments featuring narrow-bore (200–500 μm I.D.) PTFE capillary tubes with suppressed electroosmosis have been reported. In other approaches, instrumentation with array [8,9] or scanning [10] detectors along the capillary together with appropriate hardware and software for data collection and evaluation has been developed. Depending on the sophistication and availability of detectors, the programs for data evaluation permit the determination of isotachophoretic zone lengths (zone areas) for calibration and quantification, of step heights for automated zone assignment, of differential detector responses and reconstructed analogue data displays with labelled and quantified zones.

Recently, instrumentation for electrokinetic separations in fused-silica capillaries of very small I.D. (25–75 μm) became available and the first papers reporting its use for CITP appeared [11,12]. In these configurations using untreated, open-tubular capillaries, the longitudinal electroosmotic flow was found not to disturb isotachophoretic (ITP) zone formation of low-molecular-mass substances, but to make quantifica-

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tion more difficult than in classical CITP [13]. Further, it was discovered that small amounts of hydroxypropylmethylcellulose added to the buffer allowed high-resolution isotachophoretic determinations of proteins in the presence of electroosmosis [14]. Similarly, the use of coated capillaries exhibiting minimized electroosmosis for the determination of low-molecular-mass compounds [11] and proteins [15,16] was discussed. Thus, the recent advances in laboratory-made and commercial instruments for capillary electrophoresis offer broad access to CITP with very interesting features for solute monitoring, such as the use of on-column fast scanning polychrome detection [17] or on-line coupling with mass spectrometry [18].

In this study, the detector outputs of two commercial instruments, one of the old generation with a PTFE capillary (Tachophor, in an electroosmosis-free configuration) and a new, automated instrument with a fused-silica capillary (ABI 270A-HT, configuration with strong electroosmosis), were interfaced to a commercial two-channel data acquisition system featuring automatic range switching and a unique, dynamic sampling rate which provides compressed data files compared with data gathering at a constant sampling rate. Software termed UNIclip was written for CITP data evaluation based on zone length measurements of conductivity and/or UV absorption signals and the chromatographic integration software was employed for data evaluation based on peak areas of baseline-resolved UV absorption peaks. With these approaches, quantification in the presence of suppressed or active electroosmosis, and also electroosmosis combined with a vacuum generated co-flow, is compared and a discussion of the pros and cons of CITP in fused-silica capillaries is presented.

EXPERIMENTAL

Chemicals

All chemicals were of analytical-reagent or research grade. Histidine, histidine hydrochloride, sodium glutamate, glutamic acid, citric acid, lithium lactate, creatinine (CREAT), sodium chloride, tris(hydroxymethyl)aminomethane (Tris) and procaine hydrochloride were purchased from Merck (Darmstadt, Germany), γ-amino-*n*-butyric acid (GABA) and hydroxypropylmethylcellulose (HPMC) from Sigma (St. Louis, MO, USA) and ovalbumin (OVA) from chicken egg, lysozyme (LYSO) from chicken egg white and conalbumin (CAL) from Serva (Heidelberg, Germany).

Instrumentation and running conditions

Experiments with minimized electroosmosis were performed on a Tachophor 2127 analyser (LKB, Bromma, Sweden). This instrument was equipped with a 28 cm × 0.5 mm I.D. PTFE capillary and a conductivity and a UV detector (filter 277 nm) at the column end. The measurements were performed at a constant current of 150 μA. Samples were injected with a 10-μl syringe (Hamilton, Bonaduz, Switzerland). If not stated otherwise, the sample volume applied was 4 μl. The data were registered with a PM8252A two-channel strip-chart recorder (Philips, Eindhoven, Netherlands) and/or using the PC integration pack (PCIP, version 3.0) (Kontron, Zurich, Switzerland). Data storage and computations were executed on a Mandax AT 286 computer (Panatronic, Zurich, Switzerland).

Experiments in flowing solution were performed on an automated Model 270A-HT electrophoresis system (Applied Biosystems, Foster City, CA, USA) featuring fused-silica capillaries of either 50 μm I.D. (total and effective lengths 72 and 52 cm) (Applied Biosystems) or 75 μm I.D. (total and effective lengths 73.5 and 52 cm) (Polymicro Technologies, Phoenix, AZ, USA). A constant voltage of 20 kV was applied, the temperature was set to 35°C and detection was effected at 280 nm. The data were registered with the PCIP (version 3.0) (Kontron). For cationic analyses, the leader and terminator were placed in the cathodic and anodic electrolyte vials, respectively [11]. Prior to each experiment, the capillary was rinsed by vacuum aspiration (20 in.Hg; 1 in.Hg = 3386.4 Pa) with 0.1 *M* sodium hydroxide and leading buffer for 2 and 5 min, respectively. Sample injection was effected with application of a 5.0 in.Hg vacuum for 5, 10 or 20

s. This vacuum was also applied to generate a buffer co-flow during power application.

Computerized data acquisition and evaluation

The commercial PCIP together with a Mandax AT 286 computer system was used for data acquisition and raw data storage for the experiments performed on the Tachophor and the ABI 270A-HT. The PCIP features (i) automatic range switching, (ii) a dynamic sampling rate (between 0.0125 and 100 Hz) allowing sampling every 10 ms for quickly changing signals and, depending on the slope of the detector response, with a time interval between 0.01 and 80 s (this providing greatly compressed data files compared with data gathering at a constant sampling rate) and (iii) two channels for the simultaneous recording of the signals of two detectors on an equal time base (see Fig. 1).

The peak areas of UV absorbance data were determined through integration provided by the chromatographic software of the PCIP. Quantification was based on external calibration but could also have been performed using an internal standard. In ITP, quantitative results are typically obtained through zone length measurements,

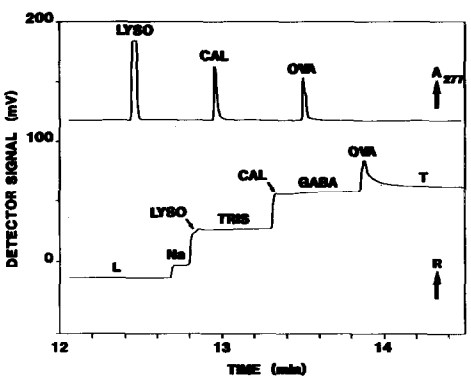

Fig. 1. Cationic CITP data for a model mixture consisting of three proteins and two low-molecular-mass spacers obtained in the Tachophor interfaced to the PCIP system. The UV absorption data (277 nm, top) and the conductivity data (expressed as resistance R, bottom) are presented. A 1-μl volume of a protein mixture containing OVA (2.6 mg/ml), LYSO (2.9 mg/ml) and CAL (2.2 mg/ml) and 1 μl of a spacer solution consisting of Tris and GABA (several mM each) were injected. L and T refer to leader and terminator, respectively (buffer system C2 in Table 1).

particularly when evaluating conductivity or potential gradient data [4,5]. Thus, a software package termed UNIclip was developed that permits the determination of zone lengths from PCIP raw data files, the establishment of linear calibration graphs based on external or internal calibration from a specified set of up to five calibration data files and quantification of a target compound in up to twenty sample runs employing the computer-stored calibration.

The basic program, which includes a shell with guidance through a menu in addition to the data, was written in Clipper (version summer 1987) (Nantucket, Los Angeles, CA, USA), whereas the programs for zone length and height determination and the graphical display were written in Turbo-C^{++} (version 1.0 in ANSI-C standard) (Borland International, Scotts Valley, CA, USA). The algorithm used for zone length determination is based on the slope between two data points, *i.e.*, the difference in signal magnitude divided by the difference in sampling time of these points. A slope higher than that of a preselected value indicates the beginning of a plateau whereas the zone end is characterized by a lower slope than the specified threshold value. Zone assignment is based on comparison of plateau values. Initially a control sample (placed as the first sample to be evaluated) is analysed for plateau values and lengths, their values being written on the screen. The target zone and, if applicable, internal standard zone are then marked, followed by carrying out the complete calibration and evaluation of data. During this procedure, zone heights of each isotachopherogram are corrected based on the relative change between the measured property of its leading zone compared with that of the control sample. Corrected plateau zone values differing by less than a specified value (typically 1–2 mV) from the marked value of the calibration zone are assigned to the zone to be evaluated. Zone lengths and heights are given in units of 0.01 s and mV, respectively. With this procedure, noisy signals might be broken up into several short plateaus of very similar zone height instead of one long zone. For reassessment of such cases, a zone height range (typically 5–20 mV) can be input and used as a second criterion for the

proper determination of the correct plateau length.

RESULTS AND DISCUSSION

Typical detector signals produced in CITP are depicted in Fig. 1. They represent (top) UV and (bottom) conductivity data of a cationic analysis of LYSO, TRIS, CAL, GABA and OVA using the Tachophor instrument together with the PCIP data acquisition system. The leader and terminator were 10 m*M* potassium acetate–acetic acid (pH 4.75) and 10 m*M* acetic acid, respectively. Depending on sample amount, UV signals represent either rectangular pulses (if sufficient sample for plateau formation is present, as is shown for LYSO) or spikes (CAL and OVA). On the other hand, conductivity data are characterized by a step-like change from the level of the leader (L) to that of the terminator (T). The CITP data format is significantly different to that observed in capillary zone electrophoresis and many forms of chromatographic analyses. Thus, CITP requires different data evaluation strategies, the chromatographic approaches being of little value for most ITP applications. The UNIclip software was developed for quantification by zone length measurements, primarily by the plateau lengths obtained with universal (*e.g.*, conductivity) detectors. It was also found to be applicable for zone lengths of UV signals. Prior to evaluating data obtained in a flowing stream, the performance of UNIclip was investigated

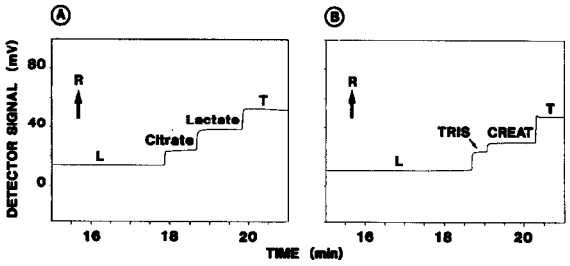

Fig. 2. CITP conductivity PCIP data expressed as resistance *R* for (A) lactate and citrate in buffer system A1 (Table I) and (B) CREAT and Tris in buffer system C1 (Table I). A 4-μl volume of a sample composed of lactate (30 m*M*), CREAT (30 m*M*), citrate (5 m*M*), Tris (5 m*M*) and 0.9% NaCl was injected. L and T refer to leader and terminator, respectively.

TABLE I

ISOTACHOPHORETIC ELECTROLYTE SYSTEMS

System	Leader	Terminator
A1	0.01 *M* histidine–0.01 *M* histidine hydrochloride–0.2% HPMC	0.005 *M* glutamic acid–0.01 *M* histidine
C1	0.01 *M* sodium glutamate–0.002 *M* glutamic acid (pH 4.95)	0.005 *M* glutamic acid
C2	0.01 *M* potassium acetate–acetic acid (pH 4.75)	0.01 *M* acetic acid

TABLE II

INSTRUMENTAL CONDITIONS

System No.	Instrument	Detector	Electroosmosis	Sample compound	Buffer system	Calibration range (m*M*)
1	Tachophor	Conductivity	Minimized	Creatinine	C1	0–30
2	Tachophor	Conductivity	Minimized	Lactate	A1	0–30
3	Tachophor	UV absorbance	Minimized	Procaine	C1	0–30
4	ABI 270A-HT	UV absorbance	Yes	Procaine	C1	0–30

with data obtained on the Tachophor. The electrolyte configurations employed and instrumental conditions are summarized in Tables I and II, respectively.

In a first approach, the use of UNIclip for the evaluation of Tachophor conductivity data of samples provided by an inter-laboratory study on accuracy and precision in CITP [19] was investigated. The cationic determination of creatinine and the anionic determination of lactate in aqueous samples containing 0.9% NaCl was undertaken. Tris and citric acid (5 m*M* each) served as internal standards, respectively. Typical isotachopherograms are presented in Fig. 2. Calibration was performed with five standard solutions having sample concentrations of 0, 2, 5, 15 and 30 m*M*. Excellent linear correlations were obtained with both internal (*i.e.*, inclusion of internal standard) and external calibration (see Systems 1 and 2 in Table III). As example, the output protocol showing the calibration and analysis data for creatinine is presented in Fig. 3. The sequence of the samples consists of the five calibrators in reversed order (data files JC1.381–301), followed by five samples (JC1.311–391). For calibration, the zero value was included. Not surprisingly for this case with little sample preparation, there is good agreement between data evaluated with (A) internal and (B) external calibration. The creatinine levels obtained were found to compare favourably (within a few per cent) with those produced in other laboratories [19]. Similar data were obtained for lactate. Thus UNIclip is shown to handle the evaluation of these data properly.

UNIclip was also employed for the evaluation of Tachophor UV absorption data, such as the cationic CITP of procaine in the electrolyte system C1 (Fig. 4A). Calibration of zone length *versus* sampled amount provided linear relationships with small intercepts when using four or five calibration points (System 3 in Table III). An alternative approach for data evaluation represents the determination of peak areas. For the very simple case of procaine, excellent correlations between peak areas determined by the PCIP integration software and sampled amount were obtained (System 3 in Table III). Thus UNIclip and PCIP software can both be employed for the evaluation of UV absorption data monitored with the Tachophor. UNIclip is capable of recognizing and assigning plateaux whose zone heights differ more than a specified value (typically 1–2 mV). The use of the chromato-

TABLE III

TYPICAL LINEAR REGRESSION ANALYSIS DATA FOR TACHOPHOR CALIBRATIONS

System No.	I.S.[a]	Evaluated zone property[b]	n[c]	Slope	y-Intercept[d]	Correlation coefficient
1	Yes	Length	5	0.115	−0.041	0.99975
	No	Length	5	240.1	−37.86	0.99993
2	Yes	Length	5	0.050	−0.018	0.99983
	No	Length	5	230.2	−89.37	0.99980
3	No	Length	5	277.6	−15.02	0.99997
	No	Length	4	278.0	−24.06	0.99997
3	No	Area	5	1.561	0.29	0.99992
	No	Area	4	1.553	0.47	0.99996

[a] Internal standard.

[b] Area determined with PCIP (mV min) and length obtained with UNIclip (0.01 s).

[c] Number of calibrators; with $n = 5$ the zero value was included.

[d] The y-intercept units for area, length with I.S. and length without I.S. determinations are mV min, ratio (dimensionless) and 0.01 s, respectively.

(A) UNIclip data: creatinine

Calibration data

File name	length	length IS	ratio	concentration
JC1.301	0	2410	0.0000000	0.0000000
JC1.321	449	2364	0.1899323	2.0000000
JC1.341	1105	2279	0.4848618	5.0000000
JC1.361	3574	2123	1.6834668	15.0000000
JC1.381	7170	2100	3.4142857	30.0000000

Results

File name	length	length IS	ratio	concentration
JC1.381	7170	2100	3.4142857	30.0591261
JC1.361	3574	2123	1.6834668	15.0017127
JC1.341	1105	2279	0.4848618	4.5743416
JC1.321	449	2364	0.1899323	2.0085764
JC1.301	0	2410	0.0000000	0.3562433
JC1.311	544	2326	0.2338779	2.3908849
JC1.331	2291	2207	1.0380607	9.3869450
JC1.351	1886	2268	0.8315697	7.5905577
JC1.371	6224	2201	2.8278055	24.9569896
JC1.391	6425	2058	3.1219631	27.5160392

(B) UNIclip data: creatinine

Calibration data

File name	zone length	concentration
JC1.301	0	0.0000000
JC1.321	449	2.0000000
JC1.341	1105	5.0000000
JC1.361	3574	15.0000000
JC1.381	7170	30.0000000

Results

File name	zone length	concentration
JC1.381	7170	30.0151598
JC1.361	3574	15.0406110
JC1.341	1105	4.7591424
JC1.321	449	2.0274116
JC1.301	0	0.1576751
JC1.311	544	2.4230129
JC1.331	2291	9.6979119
JC1.351	1886	8.0114012
JC1.371	6224	26.0758041
JC1.391	6425	26.9128131

Fig. 3. UNIclip output protocol for the calibration and determination of creatinine using (A) internal and (B) external calibration.

graphic integration software, however, is restricted to situations with zones being bracketed by non-absorbing spacers, *i.e.*, with baseline-resolved signals resembling either spikes or rectangular peaks [Fig. 1 (top) and Fig. 4A] and not step-like functions [Fig. 1 (bottom) and Fig. 2].

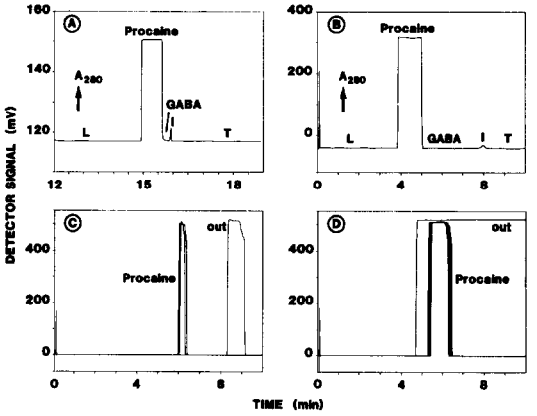

Fig. 4. UV absorption (280 nm) PCIP data for procaine in buffer system C1 using (A) the Tachophor with 2 μl of a 15 m*M* procaine sample, (B) the ABI 270A-HT with a 50 μm I.D. capillary and 20-s injection of a 15 m*M* procaine sample, (C) the ABI with a 75 μm I.D. capillary and 5-s injection of a 5 m*M* procaine sample and (D) the ABI with a 75 μm I.D. capillary and 5-s injection of a 15 m*M* procaine sample. The experiments in (A) and (B) were executed with addition of GABA for removal of the impurity (I) from the procaine peak. (C) and (D) display data with typical outliers (out) obtained with three and eight consecutive injections, respectively. For the data in (B)–(D), 100 mV corresponds to 0.1 absorbance.

Configurations exhibiting electroosmotic zone displacement are of particular interest because they can easily be implemented on both commercial and laboratory-made instrumentation. Experiments with procaine performed on the automated ABI 270A-HT employing untreated fused-silica capillaries of 50 and 75 μm I.D. (Fig. 4B) provided calibration data of equal linearity but with larger *y*-intercepts compared with those obtained with the Tachophor (Table IV). The combined application of vacuum-driven buffer flow towards the detector and power, a feature which is provided by the ABI 270A-HT, was also investigated. For one capillary (75 μm I.D.) procaine calibration data with a hydrodynamic co-flow produced through application of a 5 in.Hg vacuum during detection are presented in Table IV. Again, almost perfect linear behaviour was obtained. The combination of electroosmotic and pressure-driven flows is an attractive feature for cases with low electroosmotic flows, for dual ITP (*i.e.*, the simultaneous monitoring of cationic and anionic zone structures [11]) and to maintain the net zone displacement constant, an approach which would require a special flow control mechanism. Excellent reproducibility was achieved in the presence of electroosmosis

TABLE IV

TYPICAL LINEAR REGRESSION ANALYSIS DATA FOR PROCAINE CALIBRATIONS ON THE ABI270A-HT

System No. 4 without the use of an internal standard.

Capillary I.D. (μm)	Injection time (s)	Vacuum during detection	Evaluated zone property[a]	n^b	Slope	y-Intercept[c]	Correlation coefficient
50	10	Off	Length	3	234.0	−863.4	0.99986
50	10	Off	Area	4	15.16	5.33	0.99998
50	20	Off	Length	4	375.4	−305.3	0.99212
50	20	Off	Area	4	23.14	16.0	0.99399
75	5	Off	Length	3	349.2	−1954.8	0.98991
75	5	Off	Area	4	32.03	−12.29	0.99897
75	5	On	Area	4	5.847	−0.108	0.99976
75	15	On	Area	3	15.43	6.29	0.99994

[a] Area determined with PCIP (mV min) and length obtained with UNIclip (0.01 s).
[b] Number of calibrators (zero value was not included).
[c] The y-intercept units for area and length (without I.S.) determinations are mV min and 0.01 s, respectively.

as shown by the R.S.D. values for nine injections (Table V), the R.S.D.s of area determinations with PCIP integration software (1.3–1.4%) being lower than those based on zone lengths using UNIclip (1.6–2.2%). The same applies for the R.S.D.s of the concentration levels (Table V). Thus, based on these data, CITP in the presence of electroosmosis alone or in combination with vacuum-driven flow should constitute attractive analytical methods. This view, however, is hampered by a number of observations which are discussed in turn.

Despite automatic sampling, every set of runs was characterized with outliers, such as those seen in Fig. 4C and D, which are attributed to changes in electroosmosis. Careful conditioning did not overcome the occurrence of zones with much increased length, making quantification impossible without considering running the samples in duplicate or even triplicate, a time-consuming task. Further, with a single detector placed towards the capillary end (as in all commercial instruments), proper quantification by zone length measurements can only be performed when, during detection, the net displacement of the ITP zones remains constant. This

TABLE V

REPRODUCIBILITY DATA FOR PROCAINE IN PRESENCE OF ELECTROOSMOSIS

Capillary I.D. (μm)	Injection time (s)	Evaluated zone property[a]	n^b	Zone property		Zone concentration	
				Mean[a]	R.S.D. (%)	Mean (m*M*)	R.S.D. (%)
50	10	Length	9	3160.3	2.18	15.52	1.80
50	10	Area	9	247.43	1.41	15.43	1.43
75	5	Length	9	5058.3	1.61	15.91	1.36
75	5	Area	9	499.23	1.30	15.96	1.27

[a] Area determined with PCIP (mV min) and length obtained with UNIclip (0.01 s).
[b] Number of measurements with a sample containing about 15 m*M* procaine.

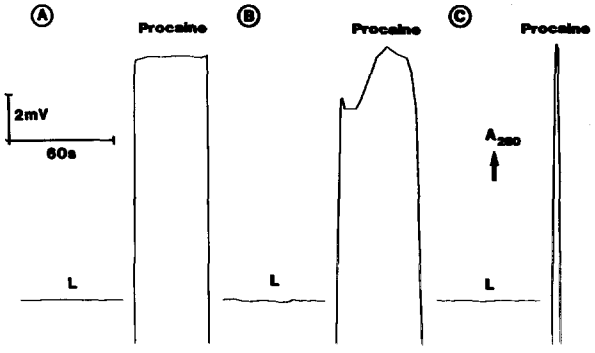

Fig. 5. UV absorption (280 nm) PCIP data with greatly expanded signal scale for leader (L) and procaine zone plateau in buffer systems C1 using (A) the Tachophor with 2 μl of a 15 m*M* procaine sample, (B) the ABI 270A-HT with a 75 μm I.D. capillary and 5-s injection of a 15 m*M* procaine sample and (C) the ABI with a 75 μm I.D. capillary, 5-s injection of a 15 m*M* procaine sample and application of 5 in.Hg vacuum during detection (beginning of co-flow after 3 min of current application).

condition, however, is difficult to meet because the electroosmotic flow is typically changing owing to the gradual change of buffer composition within the column [13]. This problem, however, is not expected in configurations of minimized electroosmosis. Second, data obtained with different capillaries showed considerable variations in noise and shape of the zone plateaux (Fig. 5). Thus, for some cases proper plateau recognition became more demanding and could not be achieved with the algorithm based on the slope between two data points, *i.e.*, the difference in signal magnitude divided by the difference in sampling time of these points. In this approach, in which a slope higher than that of a preselected value indicates the beginning of a plateau whereas the zone end is characterized by a lower slope than the specified threshold value, noisy signals were broken up into several short plateaux of very similar zone height instead of one long zone. With the use of a specified zone height range (typically 5–20 mV above and below the average plateau height) as a second condition, correct plateau lengths could be determined.

The impact of the selection of slope and height range parameters on zone length determination is shown by the data presented in Table VI. Increasing the slope parameter from 50 to 100 or 150 (lines 1, 3 and 6 of Table VI) provides longer mean zone lengths and lower R.S.D. values. A similar effect is seen with increasing height range values (compare lines 3–5). With a range value ±20 mV, equal calculated properties were obtained for all three slope values. Small deviations in zone concentrations were observed with all settings except a slope of 50 and no range value. Thus, a slope of 100 was typically chosen, the

TABLE VI

IMPACT OF SLOPE AND HEIGHT RANGE PARAMETERS ON UNICLIP ZONE LENGTH AND CONCENTRATION DETERMINATION FOR PROCAINE IN PRESENCE OF ELECTROOSMOSIS

Based on nine consecutive experiments with a 15 m*M* procaine sample, 10-s injection and a 50 μm I.D. capillary.

Slope[a] (V/s)	Height range (mV)[b]	Mean zone length (0.01 s)	R.S.D. (%)	Mean concentration (m*M*)	R.S.D. (%)
50	–	2806.0	5.21	16.61	3.90
50	20	3160.3	2.18	15.52	1.80
100	–	2920.4	3.72	15.45	2.83
100	10	3112.1	2.48	15.48	2.03
100	20	3160.3	2.18	15.52	1.80
150	–	3022.0	3.13	15.55	2.48
150	20	3160.3	2.18	15.52	1.80

[a] The value of the slope is 15 000 times higher than the actual number employed.

[b] The height range value represents the signal magnitude (mV) above and below a specified zone height, defining the range used for zone length determination.

range value being dependent on the quality of the signal. No height range value was necessary for zone length determinations of the Tachophor data whereas most sets of data obtained on the ABI 270A-HT were treated with a range value of 5–20 mV.

The difference in signal noise and shape between the two instruments is illustrated by the data presented in Fig. 5. First, it is apparent that PCIP data gathering with a dynamic sampling rate provides much smoother reponses than sampling at a fixed rate, such as 100 Hz (data not shown), making data smoothing prior to evaluation [20] obsolete. With the Tachophor, an almost perfect rectangular pulse was monitored (Fig. 5A). This, however, was rarely the case in the presence of electroosmosis and with a combination of electroosmosis and co-flow (Fig. 5B and C, respectively). It is interesting that with a slope parameter of 100 and without the use of the height range constraint, the odd-shaped procaine zone in Fig. 5B could be determined as one zone. This was not the case, however, for frequently observed signals with sharply changing noise (data not shown).

In conclusion, PCIP data gathering and automated UNIclip data evaluation provide simple, efficient and relatively inexpensive (<$3500) means for CITP in instruments featuring PTFE or fused-silica capillaries and single, on-column detectors placed towards the capillary end. The PCIP integration software can also be employed for the evaluation of baseline-resolved absorption data. This, however, is restricted to manual operation because CITP detection times are strongly dependent on the sample matrix. CITP in fused-silica capillaries with electroosmotic zone displacement along the capillary is an attractive approach for qualitative characterization of samples, such as the fractions obtained in purification [21] or body fluids received for drug screening [22]. Quantification is difficult for systems in which a significant change in electroosmosis is observed, changes which are dependent not only on the discontinuous buffer system used but also on the sample matrix [13]. Quantification is also hampered by the occurrence of outliers with a drastically different net transport across the point of detection (Fig. 4C and D), by the formation of uneven plateaux (Fig. 5B) and by the loss of certain proteins during the course of an experiment [14]. Concomitant application of a feedback-controlled, pressure- or vacuum-driven flow, however, would not only allow proper performance of dual ITP analyses [11] but would also make the net zone transport constant and therefore accurate quantification possible. The external control of the electroosmotic flow [23,24] would be an alternative to this approach.

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