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Capillary preconditioning for analysis of anions using indirect UV detection in capillary zone electrophoresis Systematic investigation of alkaline and acid prerinsing techniques by designed experiments

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Abstract

It is widely accepted in capillary zone electrophoresis, that the use of alkaline prerinse procedures can improve the reproducibility of migration times and of corrected peak areas. In this study we present a systematic investigation of alkaline and acid preconditioning procedures for anion analysis using indirect UV detection by designed experiments according to the methodology of Taguchi. Four frequently used electroosmotic flow modifiers (diethylenetriamine, hexamethonium hydroxide, tetradecyltrimethylammonium hydroxide, and hexadimethrine hydroxide) were examined. The optimized procedures were evaluated with regard to the necessary preconditioning time compared to the analysis time. Furthermore, it was demonstrated that the optimized preconditioning technique could be applied to other indirect detection based CE systems. For all examined electrolyte systems, relative standard deviations below 0.5% for migration times and below 5% for corrected peak areas ($n=20$) were achieved using automated peak integration without further manual reprocessing.

Keywords: Chemometrics; Column preconditioning; Capillary columns; Inorganic anions; Organic acids

1. Introduction

Capillary zone electrophoresis (CZE) is in the process of becoming a complementary technique to ion chromatography (IC) for the routine determination of inorganic and organic anions. For such a routine method, it is mandatory that peaks are automatically identified and integrated by commercial software packages because manual reprocessing of a large amount of data is both costly and time-

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consuming. Since any shift of migration time that cannot be compensated for by the software must be avoided, the relative standard deviations (R.S.D.) of migration times must be below 0.5% for analytes with similar mobilities.

For the determination of inorganic and organic anions by CE, modifiers are used in order to reduce, eliminate or reverse the electroosmotic flow (EOF). These modifiers adsorb on the capillary wall and dynamically coat the inner surface. If an equilibrium between the free EOF modifier in the electrolyte and that adsorbed onto the capillary wall is not reached, the velocity of the EOF, and consequently the analytes' migration time, will be irreproducible. Any processes which disturb this equilibrium will also result in irreproducible migration times. Additionally, if the sample contains compounds which can adhere to the capillary wall, e.g. surfactants, peptides or proteins, the EOF and also the migration time will be irreproducible over a set of analyses. To overcome this irreproducibility several preconditioning steps have been previously investigated and these are reviewed in Table 1. The published procedures vary considerably from a simple flush with electrolyte to complex multiple rinsing steps with alkaline or acid solution. Voltage preconditioning techniques have also been suggested – the mechanism of which is thought to act through electroadsorptive phenomena [14,16].

Each method and application seems to require a specific prerinsing procedure implying that during development of a new method the preconditioning must be also validated. To date no systematic investigations of prerinse steps have been performed for anion analysis systems using indirect UV detection. Here we present a systematic study of this topic using designed experiments according to the methodology of Taguchi [17]. Designed experiments offer the possibility of a quick and reliable evaluation of various factors within a large experimental area by simultaneously varying more than one parameter. In particular the advantage of Taguchi's approach is the reduced number of experiments necessary for considering the investigated parameters at different levels when compared to other approaches used in chemometrics. Furthermore, in Taguchi's approach the influence of a disturbance on the system is minimized without eliminating its cause. This point of view was also applied to the systematic investigations of the preconditioning. Alkaline or acidic rinsing step were considered as a disturbance of the dynamic equilibrium of the EOF modifier at the inner surface that cannot be avoided when reproducible migration time must be achieved. Subsequent flushing with electrolyte and preconditioning under separation voltage were regarded as control factors for reestablishing the original equilibrium of the modifier in the capillary.

2. Experimental

2.1. Apparatus

All experiments were carried out using an HP^{3D} capillary electrophoresis system (Hewlett-Packard, Waldbronn, Germany) at a constant temperature of 20°C. Fused-silica capillaries (50 μm and 75 μm I.D.×350 μm O.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). The length of the capillaries used, the detection wavelength and the applied voltage are given in Tables 2 and 3. For all designed experiments, a sample volume corresponding to 1% of total capillary volume [18] was hydrodynamically introduced.

2.2. Chemicals

Chemicals were purchased from various suppliers and were of analytical grade or better. The ultra-pure water used fulfilled the requirements of the valid SEMI guidelines for pure water in semiconductor processing [19]. For the preparation of anion standards, stock solutions of 25 mmol l^{-1} of the corresponding sodium salt were prepared in a class M 5.5 (US Federal Standard 209E) flow box, as were the electrolytes. Subsequent multi-anion calibration standards at 50 μmol l^{-1} and 250 nmol l^{-1} were prepared daily from single standards immediately before use in a clean room under a class M 2.5 (US Federal Standard 209E) laminar flow box.

The bromide salts of the investigated modifiers were changed to the hydroxide form in order to avoid any disturbance caused by the bromide ion, e.g. a system peak [20,21]. This was achieved by ion-exchange using the ion-exchange resin AG 1-X8

Table 1
Review of described preconditioning procedures

Electrolyte	Procedure of preconditioning	R.S.D. of migration time (%)	R.S.D. of peak area (%)	Ref.
c(Na_2CrO_4)=5 mmol l^{-1}, w(HDCr)=0.1%, pH 8.0	Purge with electrolyte	0.02–1.6 (n=N.A.)	N.A.	[1]
c(Na_2CrO_4)=5 mmol l^{-1}, c(OFM Anion-BT)= 0.5 mmol l^{-1}, pH 8.0	Rinsing with c(LiOH)=100 mmol l^{-1}, water, and electrolyte	N.A.	N.A.	[2]
c(Na_2CrO_4)=10 mmol l^{-1}, c(OFM Anion-BT)= 0.05 mmol l^{-1}, pH 8.0	Purge with electrolyte	0.1–0.3 (n=10)	0.6–1.9 (n=10)	[3]
c(Na_2CrO_4)=5 mmol l^{-1}, w(HDB)=0.001%, σ (acetonitrile)=20%, pH 11	Rinsing with electrolyte without HDB followed by rinsing with electrolyte with HDB	N.A.	N.A.	[4]
c(Na_2CrO_4)=5 mmol l^{-1}, c(TTAB)=0.5 mmol l^{-1}, pH 8.0	Purge with electrolyte	0.74–1.43	3.09–8.80	[5]
c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.6 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 7.7		0.19–0.29	3.11–6.17	
c(NDC)=2 mmol l^{-1}, c(NaOH)=5 mmol l^{-1}, c(TTAB)=0.5 mmol l^{-1}, pH 10.9		0.32–0.48 (n=18)	3.39–4.43 (n=18)	
c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.6 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 7.7	Purge with electrolyte	0.4–1.5% (n=20)	0.5–2 (n=20)	[6]
c(PMA)=1.13 mmol l^{-1}, c(TEA)=0.8 mmol l^{-1}, c(HMOH)=2.13 mmol l^{-1}, pH 7.7	Rinsing with c(HCl)=100 mmol l^{-1}, water and electrolyte	0.8 (n=N.A.)	N.A.	[7]
c(PMA)=2.5 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.6 mmol l^{-1}, c(DMOH)=0.75 mmol l^{-1}, pH 7.8–8.0	Rinsing with c(NaOH)=100 mmol l^{-1} and electrolyte	N.A.	N.A.	[8]
c(PMA)=3 mmol l^{-1}, c(DETA)=3 mmol l^{-1}, pH 7.5	Rinsing with c(NaOH)=100 mmol l^{-1}, water and electrolyte	0.3–0.54 (n=18)	0.95–4.25 (n=18)	[9]
c(NTS or NDS)=4 mmol l^{-1}, c(H_3BO_3) =100 mmol l^{-1}, c($Na_2B_4O_7$)=5 mmol l^{-1}, c(DETA)=2 mmol l^{-1}, pH 8.0	Rinsing with water, c(NaOH)=100 mmol l^{-1}, water and electrolyte	N.A.	N.A.	[10]
c(NDC)=2 mmol l^{-1}, c(NaOH)=5 mmol l^{-1}, c(TTAB)=0.5 mmol l^{-1}, pH 8.0	Purge with electrolyte	0.20–0.49 (n=18)	3.39–4.52 (n=18)	[11]
c(PDC)=5 mmol l^{-1}, c(CTAB)=0.5 mmol l^{-1}, pH 5.6	Purge with electrolyte	0.10–0.13 (n=6)	0.6–2.6 (n=6)	[12]
c(KHP)=5 mmol l^{-1}, c(MES)=50 mmol l^{-1}, c(TTAB)=100 mmol l^{-1}, pH 5.2	Rinsing with c(HCl)=100 mmol l^{-1} and electrolyte	0.18 (n=10)	2.10 (n=10)	[13]

N.A.: not available.

in the hydroxide form (Bio-Rad, Hercules, CA, USA) according to [22]. Considering the equivalent molarity of the EOF modifier, the resin was added to the modifier solution in 10% excess, stirred overnight and filtered (0.2 μm, polyamide). An aliquot of the filtrate was acidified with nitric acid and 5 ml of a solution of 5% silver nitrate was added. The ion-exchange was considered complete if no precipitation or opalescence occurred, otherwise the process was repeated by adding fresh resin to the filtrate.

The electrolytes investigated (Tables 2 and 3) were prepared daily, filtered (0.2 μm, nylon) and

Table 2
Electrolyte systems and anions under investigation for the designed experiments

	Electrolyte	Applied voltage	Signal and reference wavelength	Capillary dimensions	Anions
(I)	c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.6 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 7.8	−30 kV	350 nm/60 nm and 245 nm/10 nm	48.5 cm×50 μm	Bromide, chloride, sulfate, nitrite, nitrate, oxalate, chlorate, fluoride, formate, phosphate
(II)	c(PMA)=3 mmol l^{-1}, c(DETA)=3 mmol l^{-1}, c(Tris)=6.8 mmol l^{-1}, pH 7.5			As above	
(III)	c(CrO_3)=5 mmol l^{-1}, c(NaOH)=5 mmol l^{-1}, c(Tris)=6.5 mmol l^{-1}, c(TTAOH)=0.4 mmol l^{-1}, w(Triton X-100)=0.1%, pH 8.0	−25 kV	310 nm/10 nm and 370 nm/10 nm	64.5 cm×50 μm	Bromide, chloride, sulfate, nitrate, oxalate, chlorate, fluoride, phosphate, acetate, propionate
(IV)	c(CrO_3)=5 mmol l^{-1}, c(NaOH)=5 mmol l^{-1}, c(Tris)=7 mmol l^{-1}, w(HDOH)=0.001%, pH 8.0			As above	

degassed in an ultrasonic bath (30 min) prior to use. For each EOF modifier under investigation, a new capillary was used in order to avoid any variation caused by memory effects. A new capillary was conditioned for 30 min with 500 mmol l^{-1} NaOH followed by 30 min conditioning with the corresponding electrolyte by applying a pressure of 1 bar (10^5 Pa). The capillary was then equilibrated under separation conditions (cf. Table 2) by running ten consecutive analyses.

3. Results and discussion

It is widely accepted that a pre-analysis rinse of a fused-silica capillary with an alkaline solution [23] can improve the reproducibility of the EOF and therefore that of analyte migration times. Such a procedure can reduce or eliminate adherence of sample compounds to the capillary wall and also helps to reestablish the original equilibrium between the EOF modifier and the internal capillary surface. However, as shown in Table 1, the published procedures for preconditioning vary considerably and indicate that no standardized procedure has been evaluated to date.

The impact of each single prerinsing step to the reproducibility is only vaguely evaluated: Is an alkaline prerinsing more preferable than an acidic one, does a voltage step have a true effect, how does the order of the preconditioning steps influence the reproducibility? This inconsistency within the preconditioning procedures presents a major drawback because if each method and each application requires its own prerinsing process a standardization will not be attained. Additionally, multiple steps could unnecessarily prolong the total analysis time and reduce the sample throughput.

During our optimization of electrokinetic sample introduction [24] a pronounced shift to longer migration times was observed over a set of analyses (Fig. 1). This was more pronounced for anions with low mobilities. Consequently, automatic peak identification and integration were problematic. Further, due to the increased tailing of fluoride and phosphate peaks, the corrected peak area showed a large deviation. Rinsing the capillary with 100 mmol l^{-1} NaOH prior to each analysis improved the reproducibility of the migration time to such an extent that automatic integration was possible. Flushing with an alkaline solution completely dissociates the silanol groups on the capillary wall and at the active sites of the inner surface of the capillary the cation of the base almost completely replaces the surfactant ion used for EOF manipulation. Therefore the subsequent rinsing step with electrolyte must reestablish

Table 3
Transfer of the optimized preconditioning procedures to other electrolyte systems

	Electrolyte system and conditions	Anions under investigation
(A)	c(PMA)=2.25 mmol l^{-1}, c(NaOH)=1 mmol l^{-1}, c(BAL)=3 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, **pH 3.5**, 48.5 cm×50 μm, −20 kV, 245 nm	Pyrophosphate, oxalate, maleate, sulfosuccinate, phosphinate, phosphate, arsenate, formate, citrate
(B)	c(PMA)=2.25 mmol l^{-1}, c(NaOH)=4.5 mmol l^{-1}, c(BTP)=1.4 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, **pH 6.5**, 48.5 cm×50 μm, −20 kV, 245 nm	Bromide, chloride, sulfate, oxalate, sulfosuccinate, malonate, malate, maleate, methanesulfonate, hydroxymethanesulfonate, acetate
(C)	c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(EDA)=3 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, **pH 10.2**, 48.5 cm×50 μm, −20 kV, 245 nm	Thiosulfate, bromide, chloride, sulfate, nitrate, oxalate, sulfide, sulfite, phosphite
(D)	c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.8 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 7.8, **80.5 cm**×50 μm, −30 kV, 245 nm	Bromide, chloride, sulfate, nitrite, nitrate, oxalate, chlorate, fluoride, formate, phosphate
(E)	c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.8 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 7.8, **33 cm**×50 μm, −30 kV, 245 nm	Bromide, chloride, sulfate, nitrite, nitrate, oxalate, chlorate, fluoride, formate, phosphate
(F)	c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.8 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 7.8, **64.5 cm×75 μm,** −30 kV, 245 nm	Bromide, chloride, sulfate, nitrite, nitrate, oxalate, chlorate, fluoride, formate, phosphate
(G)	**c(CrO_3)=5 mmol l^{-1}**, c(NaOH)=5 mmol l^{-1}, c(Tris)=4.4 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 7.8, 48.5 cm×50 μm, −25 kV, 370 nm	Bromide, chloride, sulfate, nitrate, oxalate, chlorate, citrate, fluoride, formate, phosphate
(H)	**c(pAB)=20 mmol l^{-1}**, c(NaOH)=10 mmol l^{-1}, c($Ba(OH)_2$)=0.4 mmol l^{-1}, c(NH_3)=12 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 9.0, 48.5 cm×50 μm, −25 kV, 245 nm	Bromide, chloride, nitrate, sulfate, oxalate, sulfosuccinate, malonate, fluoride, maleate, succinate, phosphate, glutarate
(I)	c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.8 mmol l^{-1}, **c(DMOH)=0.75 mmol l^{-1}**, pH 7.8, 48.5 cm×50 μm, −30 kV, 245 nm	Bromide, chloride, sulfate, nitrite, nitrate, oxalate, chlorate, fluoride, formate, phosphate
(J)	c(PMA)=2.25 mmol l^{-1}, **c(TEA)=13 mmol l^{-1}**, c(HMOH)=0.75 mmol l^{-1}, pH 7.8, −30 kV, 245 nm	Bromide, chloride, sulfate, nitrite, nitrate, oxalate, chlorate, fluoride, formate, phosphate
(K)	**Electrokinetic sample introduction of a 250 nmol l^{-1} standard with −10 kV for 10 s** [24] c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.8 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 7.8, 48.5 cm x 50 μm, −30 kV, 245 nm	Bromide, chloride, sulfate, nitrite, nitrate, oxalate, chlorate, fluoride, formate, phosphate
(L)	c(CrO_3)=5 mmol l^{-1}, c(NaOH)=5 mmol l^{-1}, c(Tris)=7 mmol l^{-1}, **c(TTAOH)=0.2 mmol l^{-1}**, w(Triton X-100)=0.1%, pH 8.0, 64.5 cm×50 μm, −25 kV, 370 nm	Bromide, chloride, sulfate, nitrate, oxalate, chlorate, fluoride, phosphate, acetate, propionate
(M)	c(CrO_3)=5 mmol l^{-1}, c(NaOH)=5 mmol l^{-1}, c(Tris)=7 mmol l^{-1}, **c(TTAOH)=1.0 mmol l^{-1}**, w(Triton X-100)=0.1%, pH 8.0, 64.5 cm×50 μm, −25 kV, 370 nm	Bromide, chloride, sulfate, nitrate, oxalate, chlorate, fluoride, phosphate, acetate, propionate

Table 3. Continued

	Electrolyte system and conditions	Anions under investigation
(N)	$c(CrO_3)$=5 mmol l^{-1}, c(NaOH)=5 mmol l^{-1}, c(Tris=7 mmol l^{-1}, **c(CTAOH)=0.5 mmol l^{-1}**, w(Triton X-100)=0.1%, pH 8.0, 64.5 cm×50 μm, −25 kV, 370 nm	Bromide, chloride, sulfate, nitrate, oxalate, chlorate, fluoride, phosphate, acetate, propionate
(O)	**c(PDC)=5 mmol l^{-1}**, c(NaOH)=8.8 mmol l^{-1}, c(CTAOH)=0.5 mmol l^{-1}, pH 5.8, 64.5 cm×50 μm, −25 kV, 200 nm	Chloride, sulfate, oxalate, formate, malonate, succinate, citrate, adipate, acetate, phosphate
(P)	$c(CrO_3)$=5 mmol l^{-1}, **c(Tris)=15 mmol l^{-1}**, c(TTAOH)=0.4 mmol l^{-1}, w(Triton X-100)=0.1%, pH 8.0, 64.5 cm×50 μm, −25 kV, 370 nm	Bromide, chloride, sulfate, nitrate, oxalate, chlorate, fluoride, phosphate, acetate, propionate

the equilibrium between the cation of the base and the EOF modifier at the inner surface. For as long as no equilibrium is reached between the electrolyte cations and the dissociated silanol groups, the migration time will vary from analysis to analysis [25] which may require an extended rinsing time with electrolyte. Restoring the original equilibrium of EOF modifier at the inner surface is of critical importance. At the low surfactant concentrations typically used in anionic analysis by CZE, the association is governed by electrostatic interaction between the positively charged head group of the modifier and the negatively charged silanol groups. The surfactant ions compete with the other electrolyte cations, e.g. Na^+ and the buffering cation, for the active sites. Depending on the chemical structure of the surfactant, these modifiers may differ in their ability to adsorb to the capillary surface. Thus, varying conditioning times may be required, dependent upon the modifier used, in order to obtain a stable dynamic coating.

Since the attractive forces are weak, this equilibrium can easily be disturbed by rinsing steps which do not include an EOF modifier. Thus, it is assumed that any preconditioning procedure restores the initial condition of a 'new' capillary and the dynamic coating by the EOF modifier achieves an equilibrium condition [26]. Due to the importance of the EOF modifier in the determination of anions we investigated four common EOF modifiers (Table 2, Fig. 2) considering that some modifiers can precipitate with some coions, e.g. TTAB and PMA [27]. The opti-

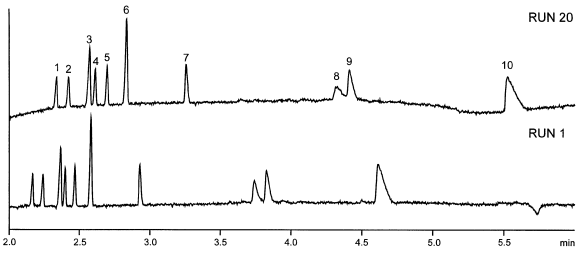

Fig. 1. Migration time shift due to simple prerinsing the capillary four times its volume prior to each analysis. Electrolyte: c(PMA)=2.25 mmol l^{-1}, c(NaOH)=6.5 mmol l^{-1}, c(TEA)=1.8 mmol l^{-1}, c(HMOH)=0.75 mmol l^{-1}, pH 7.8; injection: 250 mbar s of 50 μmol l^{-1} standard solution; capillary: 48.5 cm×50 μm; voltage: −30 kV, detection wavelength: 350 nm (signal), 245 nm (reference): (1) bromide, (2) chloride, (3) sulfate, (4) nitrite, (5) nitrate, (6) oxalate, (7) chlorate, (8) fluoride, (9) formate, (10) phosphate.

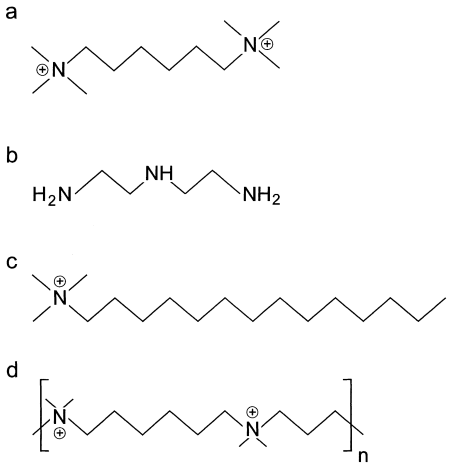

Fig. 2. Chemical structures of the used EOF modifiers: (a) hexamethonium, (b) diethylenetriamine, (c) tetradecyltrimethylammonium, (d) hexadimethrine.

mized preconditioning procedures were transferred to other systems changing the pH and the coion of the electrolyte, the capillary length and the inner diameter of the capillary as well as the alkyl chain length of the modifier used (Table 3).

The analytes used in investigating the electrolyte systems in Tables 2 and 3 were chosen such that they matched the mobility of the corresponding background electrolyte [18,28]. Inlet and outlet vials were replenished with background electrolyte prior to each separation in order to avoid any adverse effect due to electrolytic degradation [29,30]. The automated identification and integration of the peaks were performed with a method set up with the first of ten repetitions and using no further manual reprocessing of the baseline. The reproducibility of the migration time and that of the corrected peak area were evaluated as %R.S.D. [31] of ten consecutive analyses assuming normal distribution of both parameters. The values of %R.S.D. were estimated as a logarithm function and, thus, transformed into ratios of signal to noise (S/N). Consequently, the target function of the optimization was defined according to Taguchi as 'Smaller The Better' type characteristics i.e. the %R.S.D. should ideally attain a value of zero after applying the logarithm transformation with a S/N value tending to infinity. The data for each anion were evaluated separately by ANOVA (Analysis of Variances) calculations [17,32]. In defining the optimized preconditioning procedure migration time reproducibility was considered more important than peak area reproducibility. Anions with mobilities at the edges of the electrolyte coion mobility range had a more pronounced influence on the selection of the optimized parameters than those anions whose mobility nearly matched the mobility of the coion of the electrolyte [33,34]. The optimized procedure was then confirmed by 20 consecutive analyses and compared with a simple flush at 10^5 Pa with electrolyte corresponding to the replacement of four capillary volumes.

3.1. The effect of alkaline prerinsing

For the systematic investigation of an alkaline preconditioning procedure, a standardized set of designed experiments (Table 4) was defined using an L18 layout based on one factor at two levels and seven factors at three levels [17].

Upon application of the separation voltage the electrolyte and the sample in the capillary are rapidly heated which may lead to sample loss through thermal expansion and expulsion of the sample zone back out of the capillary. This can be overcome by either introducing an electrolyte plug behind the sample zone or by applying a short ramp up to the separation voltage [35]. In a direct UV detection CE system, Ross [15] recommended the use of one inlet vial for prerinsing with electrolyte, preconditioning under an applied electrical field and subsequent analysis. However, in indirect UV detection the concentration and ionic strength of the electrolyte is lower than in direct UV detection and is more sensitive to ionic depletion and possible contamination due to base carry over. Therefore, experiments using one or more electrolyte vials were performed. For the sake of clarity the following discussion will consider and refer to one-, two- and three-vial sets. In each case this refers to the number of inlet vials used after the NaOH flush. 'One set of vials' means

Table 4
Standardized set of the designed experiments for flushing the capillary with NaOH (basis of an L18 layout)

Parameters	Levels		
	1	2	3
Volume of electrolyte behind sample zone (nl)	0	2	–
Set of vials	1	2	3
Concentration of NaOH (mmol/l)	100	250	500
Flush volume of NaOH (in total volume of capillary)	1	2	3
Flush volume of electrolyte (in total volume of capillary)	2	4	6
Conditioning time under separation voltage (s)	60	180	300
Flush volume of electrolyte (in total volume of capillary)	2	4	6
Time until separation voltage is reached (min)	0.1	0.5	1.0

that the two electrolyte flushes and the voltage conditioning step were done using one vial, 'Two sets of vials' means that a different vial was used for the first electrolyte flush step, while 'three sets of vials' means that a different electrolyte vial was used for each preconditioning step. When inlet vials are exchanged some base may be transferred into the running electrolyte resulting in changes of the electrolyte composition, e.g. ionic strength and pH. NaOH was chosen for the alkali rinse because it is a constituent of the electrolyte. If the counter-ion of the base varies from that of the electrolyte an additional factor for migration time irreproducibility will be introduced [25]. This was confirmed by designed experiment using KOH, NaOH and LiOH with the first electrolyte of Table 2 (data not shown). The investigated NaOH concentrations were selected with regard to the concentration used for conditioning of a new capillary (500 mmol l^{-1}) and to the concentration often published (100 mmol l^{-1}). The rinse time with electrolyte was equivalent to a flush of at least two capillary volumes ensuring that the NaOH was completely removed from the capillary and that the time was adequate for restoring an equilibrium condition at the inner surface. Additionally, a conditioning step with an applied electrical field was examined because this should reduce the time necessary to achieve reproducible migration times by harnessing electroadsorptive phenomena [14,15].

3.1.1. Electrolyte systems using hexamethyl alkyl diammonium salts as EOF modifiers

Optimization of electrolyte system I (Table 2) resulted in the following optimum conditions:

- no electrolyte behind the sample plug
- three-vial set
- alkaline rinse of 250 mmol l^{-1} NaOH
- rinse with NaOH – two capillary volumes
- rinse with electrolyte – four capillary volumes
- 300 s conditioning under separation voltage (cf. Table 2)
- rinse with electrolyte – six capillary volumes
- ramp to separation voltage within 0.5 min.

Using a capillary of 48.5 cm total length, the above preconditioning procedure took 11 min compared to 6 min analysis time. Although the %R.S.D. values for migration time were below 0.25% (Table 5, alkaline prerinse, optimized procedure), from a practical viewpoint the preconditioning was overly time consuming. Therefore, a sub-optimum procedure was chosen which reduced the required preconditioning time, and provided adequate %R.S.D. of migration time (Table 5, alkaline prerinse, sub-optimum procedure):

- no electrolyte behind the sample plug
- three-vial set
- 250 mmol l^{-1} NaOH
- rinse with NaOH – one capillary volume
- rinse with electrolyte – four capillary volumes
- 60 s conditioning under separation voltage

Table 5
Results of the optimized preconditioning procedure for the pyromellitic acid electrolyte with hexamethonium hydroxide (Table 2, I)

	R.S.D. of migration time (%) (n=20)			
		Alkaline prerinse		Acid prerinse
	Simple flush, four capillary volumes with 10^5 Pa	Optimized procedure	Sub-optimum procedure	Optimized procedure
Bromide	2.31	0.15	0.19	0.13
Chloride	2.41	0.16	0.19	0.13
Sulfate	2.55	0.18	0.20	0.14
Nitrite	2.59	0.17	0.21	0.14
Nitrate	2.68	0.17	0.22	0.15
Oxalate	2.80	0.15	0.22	0.15
Chlorate	3.23	0.19	0.26	0.18
Fluoride	4.41	0.22	0.35	0.24
Formate	4.31	0.24	0.35	0.25
Phosphate	5.50	0.18	0.36	0.10

- rinse with electrolyte – six capillary volumes
- ramp to separation voltage within 0.5 min.

This reduced preconditioning procedure took only 7 min and resulted in R.S.D. values below 0.37% for migration time. Decreasing the preconditioning time had no significant effect on the reproducibility of corrected peak areas, in both cases below 5 %R.S.D., but slightly reduced that of the migration times because the time for restoring the equilibrium was shorter. Nevertheless, the requirements for automated peak identification and integration were fulfilled.

The sub-optimum procedure was then applied to other systems based on hexamethyl alkyl diammonium salts to examine if the preconditioning steps were independent of the pH and coion of the electrolyte, the capillary length and inner diameter as well as of the alkyl chain length of the modifier used. In electrolyte systems with a pH below 7.8 (Table 3, A and B), the reproducibility of migration times deteriorated at least 200%, from 0.7% to 1.9%. This is due to the extreme pH difference between the buffer solution and NaOH resulting in an irreproducible dissociation of the silanol groups due to the hysteresis of silanol dissociation with pH [23,36]. Reproducibility of corrected peak areas varied only slightly indicating that it was primarily depending on the automated integration of the software and the quality of the selected integration parameters within the method. For alkaline electrolytes (Table 3, C), the R.S.D. of migration times using the sub-optimum procedure were improved by least 250% from 1.9% to 0.5%, most probably due to the smaller pH difference between electrolyte and NaOH resulting in a faster compensation of the hysteresis effect. The sub-optimum preconditioning was also successfully transferred to longer and shorter capillaries as well as to capillaries with a larger internal diameter (Table 3, D–F) improving the R.S.D. of migration times by approximately three orders of magnitude to 0.4% compared to a simple flush. In a capillary with 75 μm I.D. (Table 3, F), the migration time reproducibility for phosphate was actually increased by more than 500% from 4.5% to 0.7%. Cancelling the adherence of phosphate, due to its silanophilic properties, to the inner surface [16] if the volume-to-surface ratio of the capillary is increased. Furthermore, the procedure could be applied to electrolytes with other coions and buffering components (Table 3, G and H). Again, the reproducibility was ameliorated from 0.7% to 0.4% compared to a flush four times the capillary volume, demonstrating that the preconditioning was independent of the composition of the electrolyte when using the same modifier. Changing the modifier from hexamethonium hydroxide to decamethonium hydroxide (Table 3, I), also improved the reproducibility compared to a simple flush, but only by about 30% to 0.8%. The decamethonium ion has four methylene groups more than the hexamethonium ion and therefore a slightly different adsorption mechanism could be assumed.

3.1.2. Electrolyte systems based on alkyl amines

The coion of the second electrolyte system was also pyromellitic acid (Table 2, II), but diethylenetriamine containing no Na^+ was used as the EOF modifier. The optimized parameters were determined as:

- no electrolyte behind the sample plug
- three-vial set
- 250 mmol l^{-1} NaOH
- rinse with NaOH – one capillary volume
- rinse with electrolyte – six capillary volumes
- 180 s conditioning under separation voltage
- rinse with electrolyte – six capillary volumes
- ramp to separation voltage within 0.5 min.

The reproducibility was worse compared to a flush with electrolyte only (Table 6). Possibly the prerinse steps with electrolyte could not completely replace the adsorbed Na^+. Therefore, Na^+ as well as protonated Tris and DETA might have been adsorbed to the inner capillary surface in varying concentrations causing variation in the EOF and the migration time [25].

3.1.3. Electrolyte systems based on alkyl trimethylammonium salts as EOF modifiers

In the third electrolyte system (Table 2, III) the widely used tetradecyltrimethylammonium ion was examined, but the coion had to be changed to chromate because PMA precipitates with modifiers of this type [27]. Optimization resulted in the following conditions:

- no electrolyte behind the sample plug
- three-vial set
- 250 mmol l^{-1} NaOH
- rinse with NaOH – two capillary volumes

Table 6
Results of the optimized preconditioning procedure for the pyromellitic acid electrolyte with diethylenediamine (Table 2, II)

	R.S.D. of migration time (%) (n=20)		
	Simple flush, four capillary volumes with 10^5 Pa	Alkaline prerinse	Acid prerinse
Bromide	0.29	0.46	0.12
Chloride	0.30	0.48	0.12
Sulfate	0.33	0.51	0.13
Nitrite	0.35	0.52	0.14
Nitrate	0.37	0.58	0.15
Oxalate	0.40	0.62	0.17
Chlorate	0.43	0.62	0.17
Fluoride	0.55	0.89	0.23
Formate	0.59	0.83	0.24
Phosphate	0.85	1.56	0.33

- rinse with electrolyte – six capillary volumes
- 300 s conditioning under separation voltage
- rinse with electrolyte – two capillary volumes
- ramp to separation voltage within 0.5 min.

When applying this optimized preconditioning in a capillary of 64 cm length the whole procedure took 15 min and was beyond any practical use since the analysis could be completed within 6 min. A sub-optimal procedure was considered inappropriate since the investigated procedure itself did not achieve the goal criteria. Flushing the capillary only with electrolyte resulted in a migration time reproducibility between 0.14% and 0.18% while under application of the preconditioning procedure reproducibility was 0.15% to 0.20%.

Nevertheless, the preconditioning procedure was transferred to other electrolyte systems examining the influence of the modifier concentration, type and coion (cf. Table 3). The reproducibility of corrected peak areas showed no significant improvement or deterioration supposing that the peak area primarily depended on automated peak integration by the software. For low surfactant concentrations, the migration times were at least 60% more reproducible. However, for all examined systems the reproducibility of migration time were below 0.5 %R.S.D. when only rinsing with electrolyte. From a practical viewpoint, optimizing below 0.5 %R.S.D. was considered inappropriate since the goal %R.S.D. was achieved using a simple flush for 4 min whereas the preconditioning procedure took 16 min.

The same was also true for higher modifier concentrations. Changing tetradecyltrimethylammonium to cetyltrimethylammonium hydroxide and using an electrolyte based on 2,6-pyridinedicarboxylic acid [12] reduced the migration time reproducibility from 0.05% to 0.10%. This suggests that the adsorption of alkyltrimethylammonium salts is very sensitive to an alkaline prerinse. In competing for the active adsorption sites the adsorption of Na^+ may be favored due to the smaller size of the solvated sodium compared to the bulky modifier. Consequently a longer conditioning time is required for restoring a pseudo-stationary dynamic coating.

3.1.4. Electrolyte systems based on polymeric quaternary alkyl ammonium salts as EOF modifiers

A chromate electrolyte with hexadimethrine hydroxide (Table 2, IV), a polymeric EOF modifier, was examined. The optimized parameters were determined as:

- no electrolyte behind the sample plug
- three-vial set
- 250 mmol l^{-1} NaOH
- rinse with NaOH – two capillary volumes
- rinse with electrolyte – four capillary volumes
- 60 s conditioning under separation voltage
- rinse with electrolyte – two capillary volumes
- ramp to separation voltage within 0.1 min.

Applying this procedure the reproducibility of migration times were improved by at least 250% down to 0.03% compared to a simple flush with electrolyte. However, the preconditioning procedure lasted 9 min compared to 4 min if simply flushing the capillary with electrolyte. It was therefore considered inappropriate to further improve the migration time repro-

ducibility beyond 0.5 %R.S.D. if more time is required for a sophisticated preconditioning procedure.

3.1.5. Summary of the alkaline preconditioning procedure

Only in electrolyte systems based on hexamethyl alkyl diammonium salts with an alkaline pH a multistep preconditioning procedure was superior to simple flushing with electrolyte. For the other systems investigated, an alkaline prerinsing presented no substantial improvement compared to a simple flush. Nevertheless, if an alkaline prerinsing step was carried out a subsequent voltage conditioning step significantly improved the reproducibility of migration time. Further, it was important to use different vials during the preconditioning procedure. Using 250 mmol l^{-1} NaOH gave the best reproducibilities.

3.2. The impact of an acid prerinsing

A prerinse step using HCl has been recommended to improve migration time reproducibility [7,13]; therefore, the effect of an acid preconditioning was also systematically investigated using a standardized set of designed experiments (Table 7) based on an L8 layout with seven factors at two levels [17]. The results from the alkaline prerinsing were taken into account when deciding on considered parameters which resulted in a reduced experimental array. The other considerations of the examined parameters agreed with those discussed in Section 3.1. The concentration of acid used was considered as a possible source of cross-contamination of the sample. High concentrations of acid may be difficult to flush completely out of the capillary therefore only 100 and 250 mmol l^{-1} were examined. The rinsing times with electrolyte were chosen to be at least twice as long as that of the acid ensuring complete removal of HCl. During the alkaline preconditioning it was found that a short ramp time to the separation voltage had a pronounced effect over injecting an electrolyte plug behind the sample zone. Consequently, only ramping up the separation voltage was investigated for the acid prerinse.

In ANOVA calculations for the acid preconditioning procedures, main factors could be distinguished while others had only a minor effect and could therefore be ignored, e.g. the conditioning under an applied voltage had inferior importance to the migration time. Only the main factors were used for setting up the optimized procedure. In contrast, for alkaline prerinsing procedures, the factors in the ANOVA calculation were nearly equally distributed.

3.2.1. Electrolyte systems based on hexamethyl alkyl diammonium salts as EOF modifiers

For the first electrolyte system (Table 2, I), the following procedure was found to be optimal:

- 250 mmol l^{-1} HCl
- rinse with HCl – one capillary volume
- rinse with electrolyte – six capillary volumes
- ramp to separation voltage within 0.5 min.

The electrolyte rinse was performed from a vial other than the run vial. The %R.S.D. of migration times (Table 5) and that of corrected peak areas were below 0.25% and 5%, respectively. It is presumed that during the preconditioning the proton of the acid replaces all cations from the electrolyte at the inner capillary surface due to its higher concentration. Thus, all silanol groups are protonated. Flushing with electrolyte can quickly restore the original equilib-

Table 7
Standardized set of the designed experiments for flushing the capillary with HCl (basis of an L8 layout)

Parameters	Levels	
	1	2
Set of vials	2	3
Concentration of HCl (mmol/l)	100	250
Flush volume of HCl (in total volume of capillary)	1	2
Flush volume of electrolyte (in total volume of capillary)	4	6
Conditioning time under separation voltage (s)	60	120
Flush volume of electrolyte (in total volume of capillary)	4	6
Time until separation voltage is reached (min)	0.1	0.5

rium at the capillary surface due to the fast removal of protons – the protons are not solvated by a shell but rather in common sense and do not move by a common motion, but by arrangement of the bonds of the water molecules (Grotthus transport mechanism, [37]). Additionally, the buffering component of the electrolyte also becomes protonated by the acid which further reduces the proton concentration. The electrolyte cations are electrostatically attracted by the dissociated negatively charged surface silanols. At the active sites the buffer cations have no competition.

The acid preconditioning technique resulted in better migration time reproducibility compared to the optimized alkaline wash procedure and was achieved in a shorter time (4 min instead of 11 min) with a lower electrolyte consumption (4 ml instead of 7 ml), was therefore considered to be superior (cf. Table 5).

This procedure was then applied to other systems based on hexamethyl alkyl diammonium salts as before. For all examined electrolyte systems (Table 3, A–F, I–K), the reproducibility of migration times was dramatically improved for most of the analytes to below 0.1 %R.S.D. compared with a simple electrolyte flush. Although the pH difference between HCl and electrolyte C (Table 3) was quite large no irreproducibility due to hysteresis was observed. This excellent behavior of the acid preconditioning is attributed to the transport mechanism of the protons. It is also possible to use completely buffered electrolytes, this means that no inorganic base, e.g. NaOH, is added and that the pH is only adjusted by a buffering component (Table 3, J). If such an electrolyte is used with alkaline prerinse the reproducibility is reduced because the cationic composition at the inner surface varies extremely. Therefore, the replacement of all cations from the inorganic base by those from the electrolyte takes much more time [25].

The completely buffered electrolyte (Table 3, J) shows excellent performance for migration time reproducibility ranging from 0.02% to 0.07% for 20 analyses. This is very promising for analysing long sequences, e.g. over a weekend. Assuming that the migration time shift is linear more than 140 analysis can be done before the maximum acceptable R.S.D. for migration time of 0.5% is passed. Using electrokinetic injection with electrolyte K (Table 3), a possible carry-over of the acid can be assessed not only because this system is sensitive to increased ionic strength of the sample, but also because spurious levels of chloride would be evident. No untoward effects were observed. However, one alternative for the use of HCl is propionic acid which was also tested. Its anion is out of the practical mobility range of the pyromellitic acid based electrolyte (Table 2, I) and is therefore not be detected in this system. Using propionic acid, which is a weaker acid ($pK_a = 4.87$ [38]) than HCl ($pK_a = -2$ [38]), migration time reproducibility decreased only slightly from 0.16% to 0.19% ($n = 20$).

3.2.2. Electrolyte systems based on alkyl amines as EOF modifiers

According to the main significant factors found in ANOVA calculation, the optimized procedure for electrolytes using alkyl amines as EOF modifiers (Table 2, II) resulted in the following conditions:

- 250 mmol l^{-1} HCl
- rinse with HCl – two capillary volumes
- rinse with electrolyte – four capillary volumes
- ramp to separation voltage within 0.5 min.

Two sets of electrolyte vials were used. The reproducibility of migration times was improved by at least 140% compared to a simple flush with electrolyte (Table 6). Once again the acid prerinsing technique proved superior to alkaline preconditioning or a simple flush with electrolyte.

3.2.3. Electrolyte systems based on alkyl trimethylammonium salts as EOF modifiers

As a result of the optimization of the electrolyte system based on chromate as UV absorbing coion (Table 2, III) and tetradecyltrimethylammonium hydroxide as EOF modifier, the optimum conditions were defined as:

- 250 mmol l^{-1} HCl
- rinse with HCl – one capillary volume
- rinse with electrolyte – four capillary volumes
- ramp to separation voltage within 0.1 min.

The results showed only a slight improvement compared to a simple rinse with electrolyte from 0.15% to 0.13%. This procedure was used with other electrolytes (Table 3, L–P), varying the concentration and the alkyl chain length of the modifier, the

electrolyte coion and the concentration of the buffering component The migration time reproducibility was improved for an electrolyte containing a low modifier concentration from 0.3% to 0.1% (Table 3, L) while for a higher concentration the improvement was negligible (Table 3, M–P). Quaternary ammonium ions with a long alkyl chain form a bilayer at the inner surface of the capillary where positively charged head groups are adsorbed by the deprotonated silanol groups and the long alkyl chains interact with each other by van der Waals forces. The formation of such hemimicelles also depends on the counter-ion and its concentration [39].

When the acid flush removes the hemimicelles, subsequent rinsing with electrolyte at low modifier concentrations quickly restores the original conditions because the alkylammonium ions have only to compete with the Na^+ of the electrolyte for the active sites. For higher surfactant concentrations, the ratio of modifier to Na^+ is shifted in favor of the modifier, thus, the effect of an acid prerinse becomes negligible. Consequently, at modifier concentrations reliably reversing the direction of the EOF [40–42] a rinse of four capillary volumes results in reproducible migration times. This was confirmed by the electrolyte based on 2,6-pyridinedicarboxylic acid. The used concentration of CTAOH completely reversed the EOF and further increase of its concentration had only a small influence on the EOF velocity. For completely buffered electrolytes the preconditioning procedure resulted in a decline of migration time reproducibility of at least 50%, from 0.1% to 0.4%. In electrolytes buffered by the counter-ion, the surfactant ions only compete with protonated buffer. Due to their more distributed charge, compared e.g. to Na^+, the buffer cation is not as strongly adsorbed to the active sites of the capillary surface and is easily replaced by the modifier ions having a more localized charge in its head group.

3.2.4. Electrolyte systems based on polymeric quaternary alkyl ammonium salts as EOF modifiers

Finally, for an electrolyte based on chromate with hexadimethrine hydroxide as EOF modifier (Table 2, IV), the following optimized parameters were found:

- 250 mmol l^{-1} HCl
- rinse with HCl – two capillary volumes
- rinse with electrolyte – six capillary volumes
- ramp to separation voltage within 0.5 min.

The migration time reproducibility was improved compared to simply flushing the capillary with electrolyte from 0.21% to 0.11%, but not to the same extent as for the alkaline prerinsing (0.06%). It is assumed that hexadimethrine is more readily adsorbed by deprotonated silanol groups after an alkaline rinse than by undissociated silanol groups. In addition, the further adsorption of modifier is supported by surfactant ions which are already adhered because of the van der Waals interaction between the polymeric chains. Considering the demands of a routine analysis, the reproducibility achieved by simply flushing the capillary with electrolyte is more than adequate because this preconditioning time takes only 4 min while that for the alkaline prerinse is 9 min and that for the acid prerinse is 8 min.

3.2.5. Summary of the acid preconditioning procedure

For electrolyte systems based on hexamethyl alkylammonium or alkylamines regardless of their pH, an acid preconditioning procedure was superior to an alkaline one or to simple flushing with electrolyte. In contrast to the alkaline prerinse, the voltage conditioning had no significant effect on the migration time reproducibility. Furthermore, this preconditioning is more economical through lower electrolyte consumption and allows therefore more analyses per reservoir bottle. For the other electrolytes under investigation, the acid preconditioning did not result in a significant improvement of the migration time reproducibility and therefore the more time-consuming prerinsing steps are not justified.

4. Conclusion

In several sets of experiments, designed in accordance to the methodology of Taguchi, alkaline and acid preconditioning of capillaries used with anionic electrolyte systems for indirect UV detection were systematically investigated. The examined parameters had only a minor influence on corrected peak areas R.S.D. values of better than 5% were always achieved by automated peak integration without manual reprocessing.

For electrolytes with hexamethylalkyldiammonium salts and alkylamines as EOF modifier, an acid preconditioning procedure was found to be superior to an alkaline one. Migration time reproducibility was approximately 0.02 %R.S.D. for 20 analyses. In addition the time required for the acid prerinse was shorter than that for an alkaline prerinse. Fig. 3a shows electropherograms obtained when applying the acid prerinse procedure. Fig. 3b shows the mean values of the migration times and the corresponding standard deviations expressed as Gaussian curves for the simple flush with electrolyte, the alkaline and the acid preconditioning. Lower migration time standard deviation is reflected in a narrower distribution and, therefore, better reproducibility.

When transferred to other electrolyte systems, e.g. those with modifier concentrations which reliably reverse the EOF, or completely buffered electrolyte, it turned out that a simple flush with electrolyte is superior to acid or alkaline prerinsing and, results in migration time reproducibility below 0.2%. For polymeric modifiers, the alkaline prerinsing is preferable, probably because the polymeric ions are more easily adsorbed by deprotonated silanol groups providing a stable and reproducible coating of the surface. Migration time reproducibility here was around ranges down to 0.03%.

With the use of the discussed optimized preconditioning techniques, CZE is a reliable routine technique for the determination of inorganic and organic anions. In addition, it meets the present demands of a highly automated analytical technique.

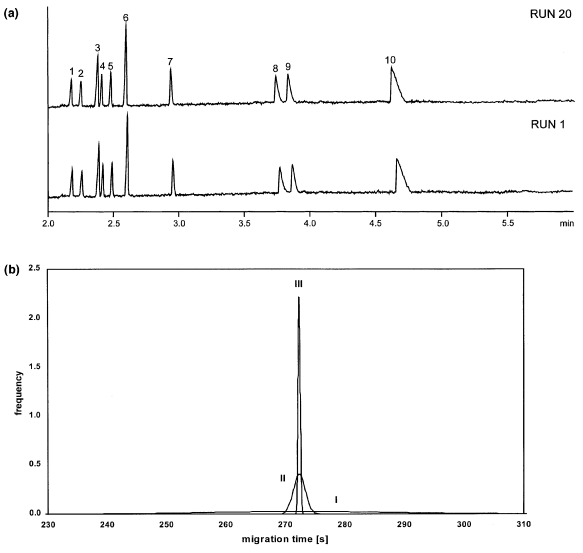

Fig. 3. Improvement of the migration time reproducibility: (a) In a set of electropherograms applying the acid preconditioning procedure: rinsing with c(HCl)=250 mmol l^{-1} for 30 s and subsequently with electrolyte for 3 min (other conditions described in Fig. 1). (b) Mean values ($\bar{x}$) and standard deviation (s) presented as Gaussian curves of phosphate for (I) simply flushing the capillary four times its volume ($\bar{x}$=272 s, s=15 s); (II) alkaline prerinsing: rinsing with c(NaOH)=250 mmol l^{-1} for 30 s, subsequently with electrolyte for 2 min, conditioning for 1 min under an applied field of −30 kV and rinsing with electrolyte for 3 min ($\bar{x}$=272 s, s=1.0 s); (III) acid prerinsing ($\bar{x}$=272 s, s=0.2 s, as above).

5. Abbreviations of used chemicals

BAL	β-alanine
BTP	1,3-bis[tris-(hydroxyethyl)-methylamino]-propane
CTAB	cetyltrimethylammonium bromide
CTAOH	cetyltrimethylammonium hydroxide
DETA	diethylenetriamine
DMOH	decamethonium hydroxide
EDA	ethylenediamine
HDB	hexadimethrine bromide
HDCr	hexadimethrine chromate
HDOH	hexadiemthrine hydroxide
HMOH	hexamethonium hydroxide
KHP	potassium hydrogenphthalate
MES	2-(N-morpholino)ethanesulfonic acid
NDC	2,6-naphthalenedicarboxylic acid
NDS	naphthalenedisulfonic acid
NTS	naphthalenetrisulfonic acid
pAB	*p*-aminobenzoic acid
PDC	2,6-pyridinedicarboxylic acid
PMA	pyromellitic acid
TEA	triethanolamine
Tris	tris(hydroxymethyl)aminomethane
TTAB	tetradecyltrimethylammonium bromide
TTAOH	tetradecyltrimethylammonium hydroxide

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