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Post-column reaction system for fluorescence detection in capillary electrophoresis

Ruohua Zhu[1], Wim Th. Kok*

Laboratory for Analytical Chemistry, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, Netherlands

Abstract

A system for post-column derivatization in capillary electrophoresis has been developed and evaluated. In this system, reagent is added through a porous tube connecting the separation capillary with a reactor capillary. The reagent solution is driven by a regulated air pressure applied on the separation capillary inlet and the reagent vessel simultaneously. The system has been applied for the fluorescence detection of amino acids using *o*-phthalaldehyde as reagent. The reactor capillary geometry and flow-rates were optimized with respect to sensitivity and separation efficiency on the basis of measured reaction rate constants. A ten-fold increase in sensitivity could be obtained by using "bubble-cell" capillaries instead of normal fused-silica capillaries for detection.

Amino acids were separated in a pH 9.7 borate buffer. Plate numbers in the order of 100 000 to 150 000 were obtained. Detection limits were typically between $2 \cdot 10^{-6}$ and $4 \cdot 10^{-6}$ mol l^{-1}, or 0.05 to 0.1 pmol injected. The method was applied to the determination of free amino acids in urine samples.

1. Introduction

In the last decade it has amply been shown that capillary electrophoresis (CE) provides an unparalleled efficiency for separations in the liquid phase. An exponentially growing number of research papers on CE has been published in the literature [1]. The bottleneck for a widespread use of CE in routine applications is now the detection. Due to the inherently small volume scale of CE, the technique still can not compete with the alternative separation method of liquid chromatography (LC) with respect to detection sensitivity. With on-column UV–Vis absorption detection the limits of detection are usually not far below 10^{-5} mol l^{-1} [2]. An improvement of the sensitivity of UV–Vis detection has been sought in the use of "extended light path" detection cells [3–6], some of which are now commercially available. Other research groups have worked on the development and application of alternative detection modes which are more suited for miniaturization, such as electrochemical detection [7–12] or laser-induced fluorescence (LIF) detection [13–17].

With the latter technique so far the lowest detection limits have been obtained, with the ultimate limit of one molecule coming within reach [18]. However, there are some obvious drawbacks of LIF detection. Apart from the high costs and limited lifetime of most lasers that give enough power and stability in the interesting wavelength range, the fact that for a particular laser only one or a few spectral lines are avail-

* Corresponding author.

[1] On leave from the Taiyuan Teachers College, Taiyuan, China.

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able is probably the most important one. Therefore, instruments using conventional incoherent light sources for the excitation in fluorescence detection are also still being developed. Although Albin et al. [16] found detection limits with different lamps of one or two orders of magnitude higher than with a laser source, later work from the group of Dovichi [19] showed that with appropriate focusing of excitation and emission light, using microscope objectives, still very low detection limits can be obtained.

Since not many compounds show strong native fluorescence, for most applications of fluorescence detection in CE derivatization of the compounds of interest will be required. As in LC, derivatization can be performed before (pre-column) or after (post-column) the separation. For the choice between the two methods similar arguments are valid as in chromatography, considering for instance the rate and optimal conditions for the reaction, the possible occurrence of multiple reaction products or the stability of the derivatives. While pre-column derivatization procedures from LC can often be applied directly in CE, the transfer of post-column technology from LC to CE is not an easy task. First, the small volume scale of CE makes the construction of a suitable post-column reaction system technically difficult. Secondly, because of the high separation efficiency of CE, the requirements in terms of peak broadening, not only in volume units but also in time units, are much more stringent than in LC.

Different approaches have been studied in the development of a post-column system for CE. One is the free-solution approach [20]. Here, the separation capillary ends in a relatively large, grounded cuvette with the reagent. In this setup the detection volume is not restricted physically but by appropriate focusing of the excitation light close to the separation capillary exit. To preserve the separation efficiency a sheath flow of reagent can be applied to sweep out the analyte zones from the illuminated detection volume. This approach seems technically simple but requires careful focusing of the light beam. Also, its application is restricted to very fast derivatization reactions, since longer reaction times can only be realized at the expense of a strong zone dilution.

In the other approach, capillaries are used as reactor and detection cell. The reagent solution can be added to the separation medium either by pressure or by electroosmotic processes. Jorgenson and co-workers developed a post-column reactor consisting of coaxial capillaries [21,22]. A small O.D. separation capillary was fixed inside a 100 μm I.D. capillary which served as the reactor. A relatively high sheath flow of reagent was driven by helium pressure. By the group of Zare [23] a cross- or tee-shaped connector was developed through which the reagent could be added with little extra zone broadening. The reagent was driven by the pressure induced by a height difference. Albin et al. [16] used electroosmosis to drive the reagent-separation buffer mixture through a reaction capillary by placing the grounded electrode after the reaction capillary. The reagent was introduced through a small gap between the separation and the reaction capillary.

Neither the addition of the reagent by pressure nor that by electroosmotic processes is trivial. In a pressure-driven system precautions must be taken to prevent laminar flow in the separation capillary, while zone broadening by laminar flow in the reaction capillary is unavoidable. In an electroosmotic-driven system the flow-rate of the reagent solution cannot simply be regulated by the diameter ratio of the separation and the reaction capillaries, as has been suggested [16]. In principle, in the absence of leakage currents, the electroosmotic volumetric flow-rate does not change with the diameter of the capillary. In a wider piece of capillary, the advantage of a larger cross-section is exactly counteracted by a lower local field strength; since the current is the same everywhere, the field strength is inversely proportional to the cross-section. Therefore, an extra independent voltage source would be required to control the reagent flow freely.

In our opinion a post-column reaction system in CE will only be used in routine analysis in the future when its operation is simple. Separation and reaction conditions should be easily controlled and capillaries easily installed and

changed. In our laboratory we have studied the design and development of such a system. To be able to work without microscope and micromanipulator, a capillary reactor system was chosen. Although realizing that the performance of fluorescence detection with post-column derivatization in terms of detection limits is mainly dependent on the optical system, in the preliminary experiments presented here an LC detector was used with only minor modifications. Experimental parameters affecting zone widths and sensitivities were studied. The derivatization of amino acids with *o*-phthalaldehyde (OPA) and 2-mercaptoethanol (2-ME) was studied as a model system.

2. Theory

Apart from the technical problems to be solved, the zone broadening caused by different reaction systems will determine the choice between them. With typical migration times of 10–15 min and plate numbers of 200 000–300 000 in CE, peak standard deviations σ are usually in the range 1–2 s. When, arbitrarily, a 25% decrease of the plate number by the post-column system is accepted, the peak-width contribution of the post-column system (σ_{PCR}) should be limited to approximately 0.5–1.0 s. In first instance this zone broadening depends on the time required for the derivatization reaction to proceed to a certain degree of completion. Although in post-column systems a reaction yield of close to 100% is not necessary, to gain as much as possible in sensitivity a high yield is desirable. Since in practice the reagent is always in excess, the reaction kinetics can be described by a pseudo-first-order rate constant k_1:

$$Y = 1 - \exp(-k_1 t_R) \quad (1)$$

where Y is the yield of the reaction and t_R the reaction time. The reaction time, which should be chosen on the basis of known or measured rate constants, is set by the volumetric flow-rate of the reaction mixture and the volume of the reactor up to the detection position.

In a pressure-driven system, the zone broadening by the laminar flow in the reaction capillary can be described by the well-known Taylor–Aris equation, which can be written as

$$\sigma^2 = \frac{d_c^2}{96D} t_R \quad (2)$$

where σ^2 is the increase in the zone variance (in s^2), d_c the diameter of the reaction capillary and D the diffusion coefficient of the (derivatized) analyte ion. In Table 1 some examples are given of the maximally allowed reaction times with different capillary diameters. In principle, the length of the reaction capillary is not of importance. If for practical reasons a certain length has to be used, the actual reaction time and the resulting zone broadening can be decreased by increasing the reagent flow. Of course, this is accompanied by dilution of the analyte zones.

It seems obvious to use very narrow reaction capillaries to confine the zone broadening. However, with a lamp fluorescence detector with limited focusing possibilities, the illuminated volume, and with that the sensitivity, will decrease when the diameter of the capillary at the detection spot is decreased.

In an electroosmotic-driven system, the osmotic flow with its flat profile will not contribute to the zone broadening. Here, however, the reaction dispersion contribution has to be considered. In general, a derivatization reaction will change the charge or size of the analyte ion, giving a difference in the electrophoretic velocity between the non-reacted analyte and the de-

Table 1
Maximum reaction times for various capillary diameters in a pressure-driven post-column reaction system

d_c (μm)	$t_{R,max}$ (s)[a]	
	$\sigma_{PCR} = 0.5$ s	$\sigma_{PCR} = 1.0$ s
25	27	108
50	7	27
75	3	12
100	2	7

[a] Calculated for a compound with $D = 7 \cdot 10^{-10}$ $m^2 s^{-1}$.

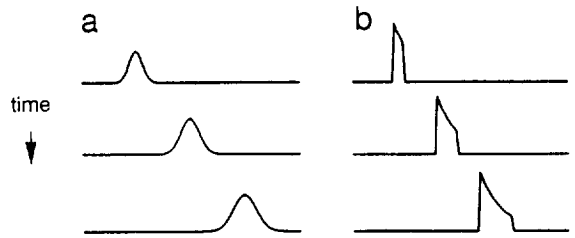

Fig. 1. The increase of the peak width in the reaction capillary by (a) laminar flow in a pressure-driven post-column reaction system and (b) reaction dispersion in a voltage-driven system.

rivatization product in the reaction capillary. In Fig. 1 the zone-broadening effect of the flow profile in pressure-driven post-column reaction systems and that of the differential migration in voltage-driven systems are shown schematically. The zone-broadening contribution of the reaction dispersion is given by

$$\sigma^2 = \frac{1}{k_1^2}\left(\frac{\Delta\mu_{eff}}{\mu_{eff,P}}\right)^2 \frac{1 - 2\exp(k_1 t_R) + \exp(2k_1 t_R) - (k_1 t_R)^2\exp(k_1 t_R)}{[\exp(k_1 t_R) - 1]^2} \quad (3)$$

where t_R is the reaction time for the analyte, $\mu_{eff,P}$ the effective mobility of the derivatization product (including the electroosmotic contribution) and $\Delta\mu_{eff}$ the difference in effective mobility between the non-reacted and the reacted species. For relatively short reaction times ($k_1 t_R \leq 1$) the zone broadening is approximately

$$\sigma^2 = \frac{1}{12}\left(\frac{\Delta\mu_{eff}}{\mu_{eff,B}}\right)^2 t_R^2 \quad (4)$$

For long reaction times ($k_1 t_R > 5$) the zone broadening approaches a constant value, given by

$$\sigma^2 = \frac{1}{k_1^2}\left(\frac{\Delta\mu_{eff}}{\mu_{eff,P}}\right)^2 \quad (5)$$

In Fig. 2 calculated zone-broadening contributions for pressure- and voltage-driven systems are shown as a function of the reaction time. A comparison of the performances of pressure- and

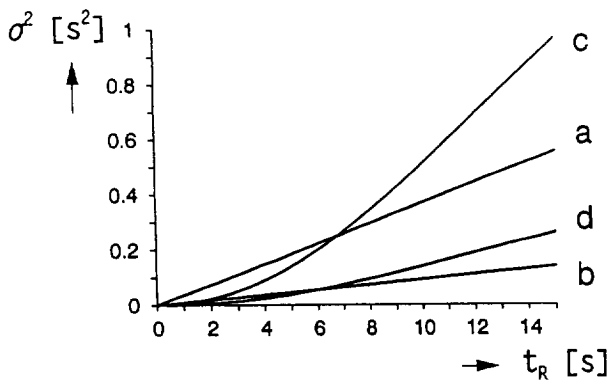

Fig. 2. Dependency of the zone broadening in a post-column reaction system on the reaction time. (a,b) Pressure-driven with a reaction capillary diameter of (a) 50 and (b) 25 μm. (c,d) Voltage-driven with a relative mobility change ($\Delta\mu/\mu$) of (c) 30% and (d) 15%.

voltage-driven post-column systems is difficult, since the first is mainly determined by the reactor diameter and the second by the chemistry of the derivatization reaction. However, as a general rule it can be stated that voltage-driven systems have an advantage when a low reaction yield ($k_1 t_R < 1$) is acceptable, while a pressure-driven system is preferable for the highest sensitivities. For the latter reason we have chosen to develop a pressure-driven system.

3. Experimental

3.1. Apparatus

Kinetic experiments were carried out with a one-line flow-injection setup, using a Spectroflow 400 HPLC pump (ABI, Ramsey, NJ, USA) and a Rheodyne injector with a 5-μl loop and coiled 0.3 mm I.D. PTFE tubing as reactor. By varying the flow-rate and the reactor length, the reaction time could be varied from 1.5 to 100 s. Detection was carried out with a Shimadzu (Tokyo, Japan) RF-530 fluorometer with a xenon lamp and a standard quartz flow cell. The detector was set at an excitation wavelength of 345 nm and an emission wavelength of 455 nm. In these experiments a strip-chart recorder and a Hewlett-Pac-

kard (Waldbronn, Germany) 3394A integrator were used.

For capillary electrophoresis, a PRINCE (Lauer Labs, Emmen, Netherlands) system was used. Samples were introduced hydrodynamically. A Fug (Rosenheim, Germany) HCN 35-35000 high-voltage source was controlled from the PRINCE. Standard fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). Bubble-cell capillaries were a gift from Hewlett-Packard. For fluorescence detection, the flow cell of the RF-530 detector was removed and replaced by a home-made capillary holder. Approximately 2 mm of the polymer coating of the capillary was burned off to make a detection window. The coating close to the detection window was painted black. Detector signals were stored and processed on a PC with an A/D converter and the Caesar 3.1 integration package for CE (Lauer Labs).

3.2. Chemicals and solutions

OPA and sodium tetraborate were obtained from Merck (Darmstadt, Germany), other chemicals from Aldrich (Brussel, Belgium). All chemicals were of analytical-grade purity and were used without further purification. Solutions were prepared with doubly distilled water.

For the electrophoretic separations sodium tetraborate buffers were used, adjusted to the desired pH with sodium hydroxide. A 25 mg/ml stock solution of OPA in methanol was stored at 4°C in the dark. The reagent solution used in CE experiments was prepared fresh each day by diluting 1.5 ml of the OPA stock solution, 18 μl of mercaptoethanol, 12.5 ml of a 0.1 mol l^{-1} borax buffer (pH 10) and 5 ml methanol to 25 ml with water. All solutions were degassed with helium before use.

3.3. The post-column reaction system

In Fig. 3 the setup used for post-column reaction detection is shown schematically. A reagent vessel, with a total volume of approximately 1 ml, was manufactured from Kel-F. The separation and reaction capillaries could be fixed

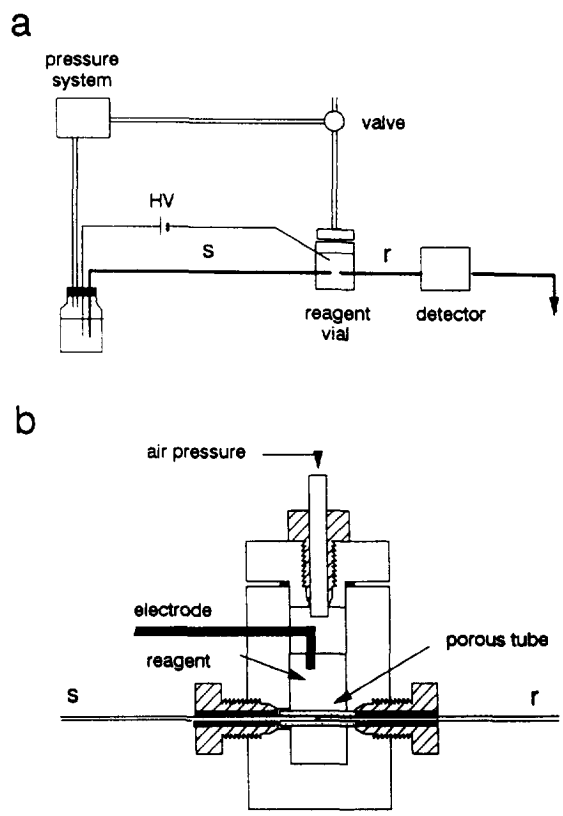

Fig. 3. Scheme of the post-column reaction system (a) with cross-section of the reagent vessel (b, not on scale). HV: high-voltage source; s: separation capillary; r: reaction capillary.

with fingertight fittings into a tube of porous PTFE (3.5 mm O.D., 0.35 mm I.D.) inside the reagent vessel, with a minimal gap between them. Reagent was driven by air pressure through the porous tube into the gap between the two capillaries. The pressure on the air-tight vessel was provided by the injection system of the PRINCE apparatus. Connections were made from 1 mm I.D. PEEK tubing. To prevent Poisseuille flow in the separation capillary, the pressure was applied simultaneously on the reaction vessel and on the inlet vial of the electrophoresis capillary during the separation. Furthermore, the inlet vial and reaction vessel are positioned at the same height.

To provide the possibility of hydrodynamic injection, an electrically actuated low-volume

gas-valve, controlled from the PRINCE, was inserted in the air-pressure line. Before injection the gas valve was switched, so that the connection to the PRINCE is closed, and the connection to the reagent vessel opened to ambient air. During analysis the valve connected the reagent vessel with the PRINCE. Because of the change of the pressurized air volume by the instrumental modification, the pressure system had to be recalibrated before use.

A platinum wire through the vessel wall, in contact with the reagent solution, served as the (grounded) electrode for the high voltage.

4. Results and discussion

4.1. Kinetics of the OPA reaction

Kinetic data on the OPA reaction of the common amino acids have been published by Svedas et al. [24] and Meyer et al. [25]. It was shown that the reaction is second order in the amino acid and OPA concentrations, with rate constants between 20 and 100 $mol^{-1}\,l\,s^{-1}$ for most amino acids. For a further study of the kinetics and rate optimization, flow-injection experiments have been performed with some exemplary amino acids. A small (5 μl) sample plug was introduced in a reagent solution stream, and the reaction time was varied by changing the flow-rate and/or the length of the reaction coil. Fluorescence signals were recorded. The peak areas obtained were corrected for the varying hold-up time in the detector cell by division through the measured flow-rate. Since in all experiments the reagent concentration was in large excess, the proceeding of the reaction could be described as a pseudo-first-order process, with a yield as described by Eq. 1. When A' is the corrected peak area at a certain reaction time and A'_{max} the maximum value obtained at long reaction times, a plot of $\ln(1 - A/A_{max})$ versus t_R should give a straight line through the origin with a slope equal to $-k_1$. Fig. 4 gives examples of the kinetic curves and the linearized plots obtained. Only for lysine this approach did not work, due to the relatively fast degradation

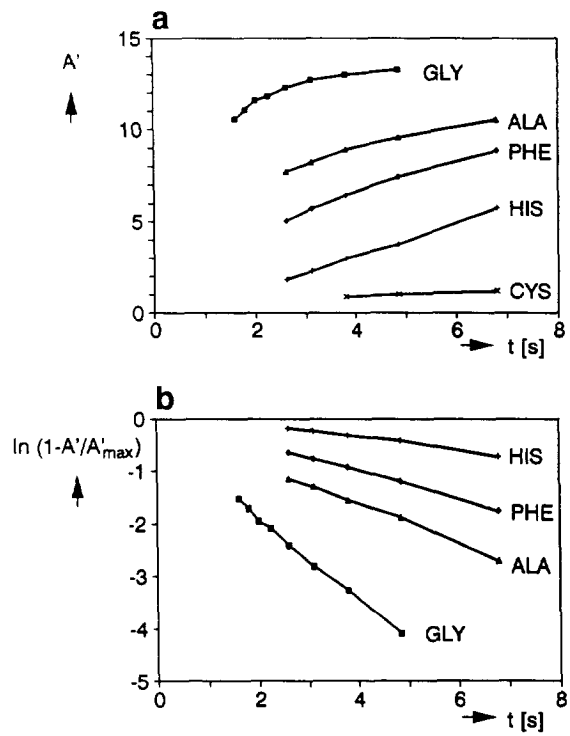

Fig. 4. Measurement of the kinetics of the OPA reaction of amino acids. (a) Kinetic curves; (b) linearized plots. Used was 5 mmol l^{-1} OPA and 2-ME in a pH 10 solution; 5 μl of 10^{-4} mol l^{-1} of amino acids injected.

of the reaction product. For all other amino acids examined the pseudo-first-order rate constant was proportional to the OPA concentration in the range from 1 to 10 mmol l^{-1}. Calculated second-order rate constants showed a fair agreement with literature values, except for glycine, for which a higher rate has been reported [24], and for cysteine, which reacted much slower in our experiments. At OPA concentrations higher than 10 mmol l^{-1} the maximum fluorescence intensities were decreased.

The 2-ME concentration appeared to be not critical within the range 1–5 mmol l^{-1}. At higher 2-ME concentrations the reaction rates and the maximum fluorescence intensities dropped. Optimum pH values were between 9 and 10, depending on the amino acid tested. The addition of up to 20% (v/v) methanol or ethanol to the reagent mixture increased the rate constants slightly. The addition of sodium dodecylsulphate

(SDS) to the reagent did not influence the rate constants nor the fluorescence intensities significantly. However, SDS appeared to stabilize the OPA–lysine reaction product when the pH was 9 or lower, so that kinetic analysis became possible. With an SDS concentration of 20 mmol l^{-1}, a relatively high rate constant (93 $mol^{-1}\,l\,s^{-1}$) was measured for lysine at a pH of 9. In Table 2 measured rate constants for a number of amino acids are given.

Acknowledging that the (first-order) rate constants as given in Table 2 represent approximately optimum conditions, it is clear that for most amino acids a reaction time of 5–10 s is required in a post-column system to obtain optimal sensitivity. From Table 1 it can be seen that in that case the reaction capillary diameter should not exceed 50 μm.

4.2. Reactor capillary geometry and pressure control

In the choice of the reaction capillary diameter the contradicting requirements in terms of zone broadening and sensitivity should be met. The relative sensitivities of the detector in use obtained with different capillaries were determined by measuring the fluorescence of 10^{-4} to $5 \cdot 10^{-4}$ mol-l^{-1} quinine sulphate solutions in 0.05 mol l^{-1} sulphuric acid. Large plugs of the quinine sulphate solution were injected in a pressure-driven flow to eliminate effects of sample volume and dilution. Conventional capillaries with a 2-mm detection window and Hewlett-Packard "bubble-cell" capillaries were tested. The results are given in Fig. 5. The data in this figure are only indicative; especially with conventional capillaries the variance of the sensitivity was large due to small changes in the exact position of the capillary in the capillary holder. Still, it was clear that with the bubble-cell capillaries approximately a ten-fold increase of the sensitivity could be obtained compared to the conventional capillaries with the same inner diameter. Therefore, in further experiments a 50-μm bubble-cell capillary was used as reactor.

Table 2
Pseudo-first-order and second-order rate constants of the reaction of OPA with selected amino acids at pH 10

Amino acid	k_1 $(s^{-1})^{a,b}$	k_2 $(mol^{-1}\,l\,s^{-1})^{b}$
Gly	0.77	153
Ala	0.36	72
Phe	0.26	52
His	0.15	31
Cys	0.02	4
Lys[c]	0.46	93

[a] [OPA] = 5 mmol l^{-1}.
[b] [2-ME] = 5 mmol l^{-1}.
[c] pH 9, 20 mmol l^{-1} SDS added.

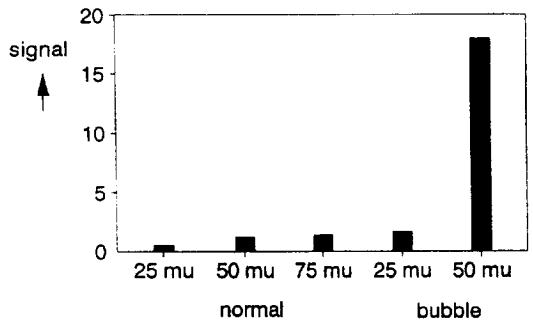

Fig. 5. Relative fluorescence intensities of quinine sulphate solutions obtained with various normal and "bubble-cell" fused-silica capillaries.

Since in the system as developed the air pressure used to drive the reagent solution is applied simultaneously on the reaction vessel and the capillary inlet, the flat electroosmotic flow profile in the separation capillary is expected not to be affected. We have found that this expectation comes true except when very wide (100 μm) separation capillaries were combined with narrow (25 or 50 μm) reaction capillaries and high reagent flow-rates. In this case migration times shorter than expected and increased zone widths were found. This was probably caused by the flow resistance of the porous tube over the connection between the two capillaries. The resulting pressure drop over the separation capillary causes a laminar flow superimposed on the electroosmotic flow in the direction of the reagent vessel. With 50 or 75 μm I.D. separation capillaries migration times were found to be not dependent on the reagent flow-

rate, so that the laminar flow in the separation capillary is apparently negligible here. In further experiments generally 75 μm I.D. separation capillaries were used.

Since there is no electric field in the reaction capillary, the flow-rate in it can be described by Poisseuille's law. The total flow is comprised of the reagent flow and the electroosmotic flow from the separation capillary. Therefore, a minimum pressure P_{min} is required before the reagent solution can enter the reaction capillary (see Fig. 6). When a lower pressure is applied, the electrophoretic buffer will partly flow into the reaction vessel. When it is assumed that there is no electroosmosis in the porous tube, P_{min} is the pressure required to drive the electroosmotic flow from the separation capillary through the reaction capillary:

$$P_{min} = 32\eta\mu_{os}\Delta V\frac{L_2 d_1^2}{L_1 d_2^4} \quad (6)$$

where η is the solution viscosity, μ_{os} the electroosmotic mobility, ΔV the applied voltage and L_1, L_2, d_1 and d_2 the lengths and diameters of the separation and reaction capillaries, respectively. Experimental and calculated values of P_{min} were in fair agreement. For instance, using a 90 cm × 75 μm separation capillary with an applied voltage of 20 kV and a 22 cm × 50 μm reaction capillary, the calculated value of P_{min} was 94 mbar, while the lowest pressure that yielded fluorescence signals in practice was 90 mbar. This shows that the electroosmotic effects in the porous PTFE tube are indeed small.

With respect to sensitivity it would be best to use a low reagent flow-rate to minimize zone dilution, and to adapt the reactor length according to the desired reaction time. However, with the detector used in this study, at least 11 cm of reaction capillary was required up to the detection window. Therefore, relatively large reagent flows were used. As shown in Fig. 7, an increase in the reagent flow results in an increase of the observed plate numbers and a decrease in the peak area. In further experiments, a pressure of 180 mbar was used, giving a buffer/reagent flow ratio of approximately 1:1 and a reaction time of 15 s. Under these conditions plate numbers were typically between 110 000 and 140 000 for negatively charged amino acids.

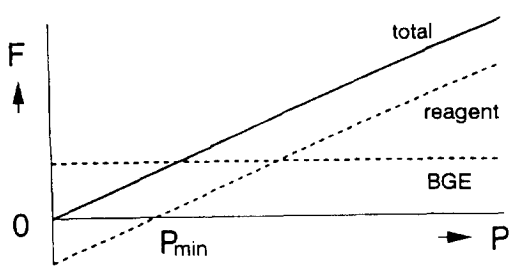

Fig. 6. Theoretical dependency of the reagent flow-rate on the applied pressure.

4.3. Separation of amino acids

Borate buffers were examined as background electrolyte (BGE) for the separation of the common amino acids. It was found that the plate numbers obtained depended on the concentration of the buffer; the highest efficiency was obtained with a buffer concentration of 0.015 mol l^{-1}. The influence of the buffer pH on the

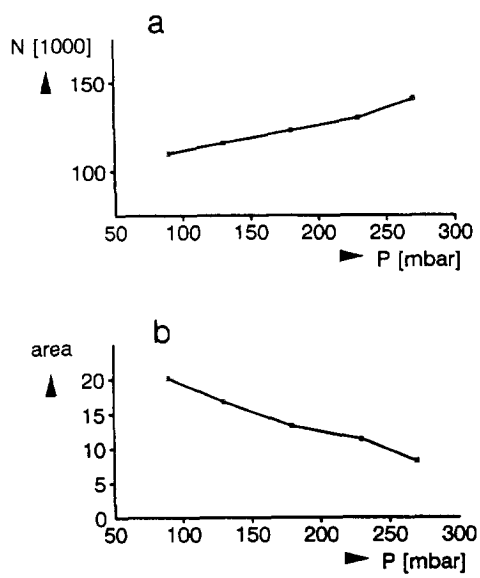

Fig. 7. Influence of the applied pressure on the plate number (a) and area (b) of the Ala peak. Separation capillary: 90 cm × 75 μm; reaction capillary: 22/11 cm × 50 μm. Applied voltage 20 kV.

migration times was studied, with pH values between 9.3 and 10. Although changes of the selectivity and of the order of the peaks with the pH were found, a complete separation of all natural primary amino acids could not be realized at any pH in this simple buffer system. For instance, at pH 9.7 Ala could not be separated from Ile and Met not from Tyr. Still, at a pH of 9.7 the major amino acids found in urine samples could be separated. The peak of Lys showed tailing. Lys and Arg could be separated by addition of 0.01 mol l^{-1} SDS to the buffer, while the separation of the other amino acids was not affected by SDS. The resulting electropherogram is shown in Fig. 8a. For comparison, amino acids

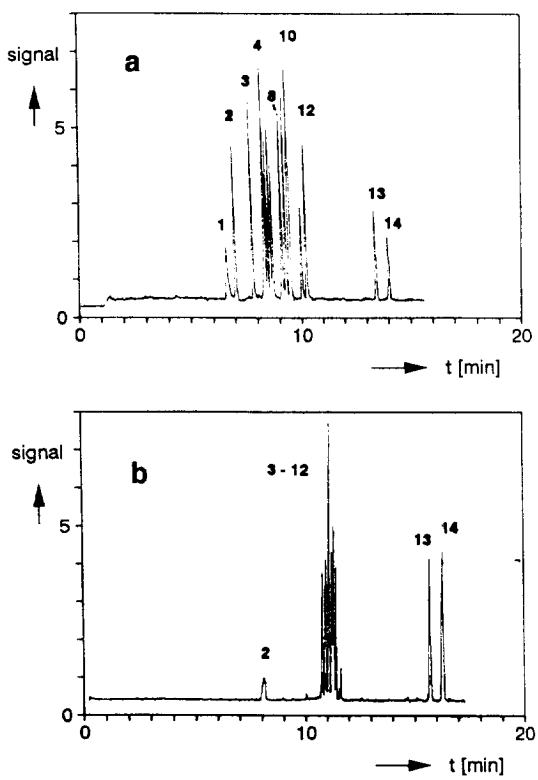

Fig. 8. Electropherograms of an amino acid standard mixture with (a) post-column or (b) pre-column derivatization with OPA. BGE: 0.015 mol l^{-1} borate, 0.01 mol l^{-1} SDS, pH 9.7. Applied pressure 180 mbar. Other conditions as in Fig. 7. Concentration of amino acids $5 \cdot 10^{-5}$ mol l^{-1} each. Peaks: 1 = Lys; 2 = Arg; 3 = AIBA; 4 = Ala; 5 = Leu; 6 = Val; 7 = Trp; 8 = Gly; 9 = Phe; 10 = Met; 11 = Thr; 12 = Ser; 13 = Glu; 14 = Asp.

derivatized with OPA shortly before the separation were analyzed under otherwise similar experimental conditions (Fig. 8b). It is clear that the derivatization has a considerable influence on the mobilities of the amino acids, and that a large part of the separation selectivity is lost with pre-column derivatization. It should be noted, however, that the separation conditions had been optimized for the underivatized compounds. Note that the unstable lysine derivative is not visible in the electropherogram. For the other amino acids the sensitivities obtained with pre- or post-column derivatization were of the same order of magnitude.

With the post-column reaction system, using 10^{-4} mol l^{-1} standard mixtures of amino acids, retention times were found to be reproducible within 0.5% and peak areas within 4.5% ($n = 7$). Peak areas were linear with the sample concentration in the range from $5 \cdot 10^{-6}$ to 10^{-3} mol l^{-1}, although at concentrations higher than $2 \cdot 10^{-4}$ mol l^{-1} peak distortion by overloading became visible. Detection limits, calculated for a signal-to-noise ratio of 2, ranged from $2 \cdot 10^{-6}$ to $4 \cdot 10^{-6}$ mol l^{-1}, or 0.05 to 0.1 pmol injected.

Urine samples could be analyzed without any sample pretreatment except dilution (1 to 10) with distilled water. In Fig. 9 electropherograms are shown of a urine sample and a standard mixture of amino acids which are usually present in urine samples in appreciable concentrations ($>5 \cdot 10^{-5}$ mol l^{-1}). Peaks in the electropherograms of the urine samples could be identified on basis of their migration times and by spiking with a standard mixture. The amino acid concentrations found in urine samples from healthy volunteers were within the normal range [26]. In all urine electropherograms a large, unidentified peak appeared at a migration time of 11.3 min.

5. Conclusions

In this study it has been shown that with a relatively simple pressure-driven post-column reaction system for CE, plate numbers in the order of 100 000 to 150 000 can be obtained. By applying a regulated air pressure on the capillary

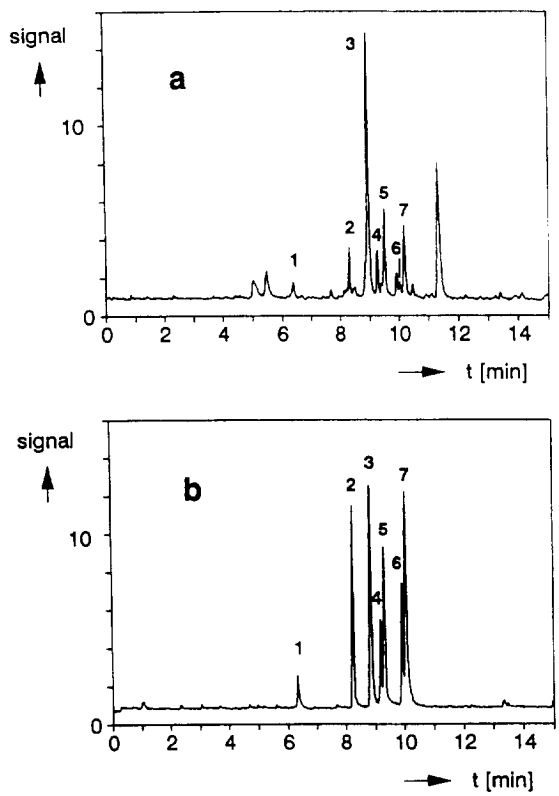

Fig. 9. Electropherograms of (a) a urine sample diluted 1:10, and (b) a 10^{-4} mol l^{-1} standard mixture of amino acids. Experimental conditions as in Fig. 8. Peaks: 1 = Lys; 2 = Ala; 3 = Gly; 4 = His; 5 = Tyr; 6 = Thr; 7 = Ser.

inlet and the reagent vessel simultaneously during electrophoresis, the reagent flow-rate could be controlled without introducing laminar flow in the separation capillary. Since the experimental results followed the theoretical predictions closely, the conditions to obtain the optimal compromise between sensitivity on one hand and zone broadening on the other can easily be found.

For the fast reaction between amino acids and OPA studied in this work, a short reaction capillary, giving reaction times of 5–10 s, would have been sufficient. Since with the modified LC detector as used here a minimum capillary length is required, part of the sensitivity had to be traded in to keep the zone broadening within limits. Improvements in this respect can be expected with an optical system specially designed for CE, possibly based on fibre optics.

Detection limits (LODs) found in our work were in the order of 50 to 100 fmol injected both with post-column and with pre-column derivatization. These LOD values are approximately one order of magnitude higher than those reported for OPA derivatives by Albin et al. [16], who also used a xenon lamp as excitation source. Therefore, we believe that an appreciable gain in sensitivity can still be obtained by an improvement of the optical system. Work in this direction is now being carried out in our laboratory.

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