Journal of Chromatography, 516 (1990) 223–231
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 575

Influence of buffer concentration, capillary internal diameter and forced convection on resolution in capillary zone electrophoresis

HENRIK T. RASMUSSEN and HAROLD M. McNAIR*
Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 (U.S.A.)

ABSTRACT

The relative velocity difference ($\Delta U/U_{av}$) of two zones, separated by capillary zone electrophoresis, increased with increasing buffer concentration, but remained constant for a given concentration regardless of electric field strength. For diffusion-limited band broadening, the increase in $\Delta U/U_{av}$ offset a decrease in migration velocity to provide slightly better resolution. In practice, however, additional dispersion occurred as a result of Joule heating, especially when concentrated buffers, high electric field strengths and/or capillaries with large internal diameters were employed. To improve efficiency, under such conditions, forced air convection was investigated as a means for dissipating some of the Joule heat generated. In 100-μm capillaries, forced convection increased efficiency from 190 000 ± 3.1% relative standard deviation (R.S.D.) to 226 000 ± 3.3% R.S.D. theoretical plates. For comparison, 264 000 theoretical plates were observed in 50-μm capillaries under similar operating conditions. In the latter case the improved efficiency is, however, obtained at the expense of sample capacity.

INTRODUCTION

By Giddings[1] definition, the resolution (R_s) between two species migrating at different linear velocities is given as:

$$R_s = \frac{N^{1/2}}{4} \frac{\Delta U}{U_{av}} \tag{1}$$

where N is the number of theoretical plates, and $\Delta U/U_{av}$ the relative velocity difference between the two components. This relative velocity difference may, for capillary zone electrophoresis (CZE), be expressed[2] as:

$$\Delta U/U_{av} = \frac{v_{el.1} - v_{el.2}}{v_{el.av} + v_{eo}} \tag{2}$$

0021-9673/90/$03.50

where $v_{el.1}$ and $v_{el.2}$ are the electrophoretic velocities of the two species being separated, $v_{el.av}$ their average electrophoretic velocity and v_{eo} the velocity resulting from electroosmosis; v_{el} and v_{eo} may be further defined as:

$$v_{el} = \mu_{el}E \qquad \text{and} \qquad v_{eo} = \mu_{eo}E \tag{3}$$

where E is the electric field strength, μ_{el} the electrophoretic mobility and μ_{eo} the coefficient of electroosmotic flow. Combination of eqns. 1–3 yields:

$$R_s = \frac{N^{1/2}}{4} \frac{\mu_{el.1} - \mu_{el.2}}{\mu_{el.av} + \mu_{eo}} \tag{4}$$

which predicts that optimization of resolution is contingent on controlling the parameters that control μ_{eo} and μ_{el}.

Expressions for μ_{eo} and μ_{el} may be given as[3]:

$$\mu_{eo} = \varepsilon\zeta/4\pi\eta \tag{5}$$

and

$$\mu_{el} = ze/6\pi\eta r \tag{6}$$

where ε is the dielectric constant of the buffer employed, ζ the zeta potential at the buffer–capillary interface, η the buffer viscosity, z the charge of the analyte, e the charge of an electron and r the analyte's hydrodynamic radius.

As seen from eqns. 4 and 5, resolution in CZE may be optimized by changing the zeta potential. This may be achieved by varying the buffer pH[4,5], by coating the capillary[6,7], by means of buffer additives[5,8] or, as will be discussed in this paper, by changing the buffer concentration[9].

It should be noted from eqn. 4 however, that for resolution to be optimized, the procedure employed to control μ_{eo} must not significantly reduce N. Due to the flat flow profile provided by electroosmotic flow[10], N may, in the absence of Joule heating and extra-column band broadening, be expressed as[2]:

$$N = (\mu_{eo} + \mu_{el})V/2D \tag{7}$$

where V is the applied voltage used to effect the separation and D the molecular diffusion coefficient of the analyte.

In the presence of Joule heating however, a radial viscosity gradient is established inside the capillary and as a result N may be decreased considerably[2,11–14]. Temperature additionally affects μ_{el}, μ_{eo} and D, and as such the degree of Joule heating becomes an important parameter in determining resolution. The Joule heat generated increases as capillary I.D., or electric-field strength is increased. To provide a viable comparison of resolution for different buffer concentrations, the influence of these parameters must therefore also be examined.

This work focuses on determining the influence of buffer concentration on resolution and characterizes forced air convection as a method for controlling Joule heating.

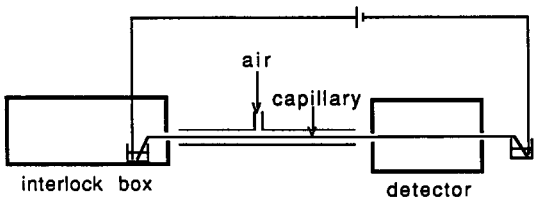

Fig. 1. Schematic of the apparatus for CZE employing forced convection.

EXPERIMENTAL

CZE was performed in 1 m × 100 μm I.D. (Chrompack, Raritan, NJ, U.S.A.) and 1 m × 50 μm I.D. (Polymicro, Phoenix, AZ, U.S.A.) fused-silica capillaries, using 0.01, 0.02 and 0.05 *M* Na_2HPO_4, pH 7.00. To determine the influence of electric field strength on electroosmotic flow, phenol was dissolved in each operating buffer, introduced into the capillaries electrokinetically (+3 kV/5 s) and analyzed at operating voltages of +10–+25 kV. In experiments where electrophoretic mobilities were additionally required, sodium toluenesulfonate (STS) was added to the above samples.

Voltages required for sample introduction and electrokinetic migration were provided by a Spellman Model RHR 30 high-voltage power supply (Spellman, Plainview, NY, U.S.A.). On capillary UV detection at 254 nm was effected 80 cm from the point of sample introduction, using an ISCO Model CV4 capillary electrophoresis absorbance detector (ISCO, Lincoln, NE, U.S.A.). To evaluate the effect of forced air convection, the capillary was inserted into a 50 cm × 8 mm I.D. glass tube and air introduced through a sidearm in the tube at 3.5 l/min (Fig. 1).

RESULTS AND DISCUSSION

Due to the dependence of resolution on each of the operating parameters in CZE, optimization of resolution must be discussed with respect to all the variables. Consequently, to determine the influence of buffer concentration on resolution, the influence of capillary internal diameter and electric field strength must be evaluated. As shown by Boček *et al.*[13], the relationship between Joule heating and μ_{eo} may be determined for a given capillary diameter by measuring μ_{eo} as a function of electric field strength.

In this study, μ_{eo} was determined from the migration times (t_R) of phenol, using the equation[15]:

$$\mu_{eo} = L/t_{R(phenol)}E \tag{8}$$

where L is the distance along the capillary from the point of sample introduction to the detector (80 cm). The use of phenol as a μ_{eo} marker has been documented previously[4]. Phenol has a pK_a of 10.0 at 25°C, and may thus be considered neutral at a pH of 7.00.

The influence of buffer concentration and electric field strength on μ_{eo} in a 50 μm capillary is shown in Fig. 2. The use of more concentrated Na_2HPO_4 buffers decreased μ_{eo} (refs. 7 and 9). Additionally, as indicated by the increase in μ_{eo} with increasing

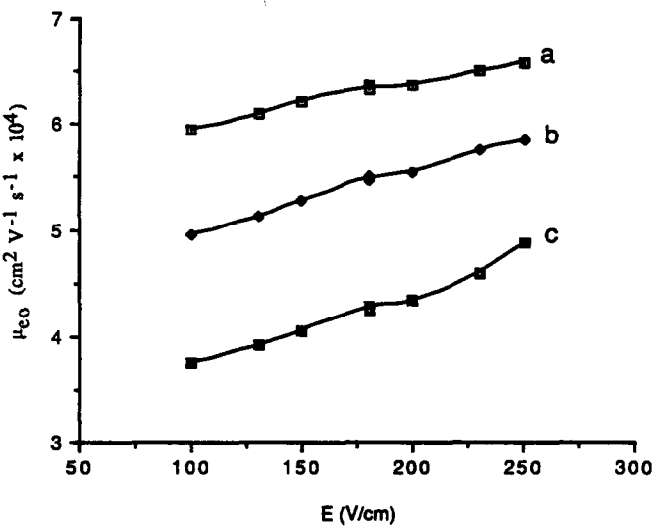

Fig. 2. Influence of buffer concentration and electric field strength (E) on the coefficient of electroosmotic flow (μ_{eo}) in 50-μm capillaries[13]. Na_2HPO_4 concentrations: a = 0.01 M; b = 0.02 M; c = 0.05 M.

electric field strength, Joule heating of the buffers occurred, which is in agreement with the results reported by Boček *et al.*[13]. In 100-μm capillaries (Fig. 3) the same relationship between buffer concentration and μ_{eo} was observed at lower electric field strengths. However, as expected, Joule heating was more pronounced in the larger capillary, especially when concentrated buffers were employed; as a result μ_{eo} increased markedly with electric field strength.

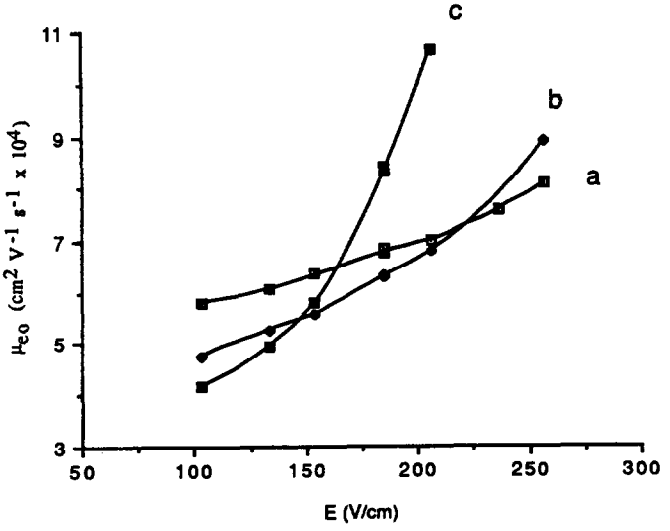

Fig. 3. Influence of buffer concentration and electric field strength (E) on the coefficient of electroosmotic flow (μ_{eo}) in 100-μm capillaries[13]. Na_2HPO_4 concentrations: a = 0.01 M; b = 0.02 M; c = 0.05 M.

As can be seen from eqns. 4 and 7, the effects observed in Figs. 2 and 3 make it difficult to determine the optimum experimental conditions for CZE. From eqn. 4, μ_{eo} is seen to be an important parameter in determining the relative velocity difference. From eqn. 7, the increase in both the applied voltage and μ_{eo} is predicted to have a favorable influence on N. The improvement in diffusion-limited efficiency is, however, potentially offset by the greater degree of Joule heating.

To better understand the influence of μ_{eo} and Joule heating on resolution, the various operating parameters were evaluated with respect to separation efficiency and the relative velocity difference. The relative velocity difference $(\mu_{el.1} - \mu_{el.2})/(\mu_{el.av} + \mu_{eo})$ was determined from the separation of phenol and STS. By virtue of its negative charge, toluenesulfonate eluted after phenol and its electrophoretic mobility was designated $\mu_{el.2}$; $\mu_{el.1}$ corresponds to the electrophoretic mobility of phenol and was assumed to be zero. Since the total linear velocity (v) of STS is governed by both electrophoresis and electroosmosis, its migration time is given as:

$$t_R = L/v = L/(-\mu_{el} + \mu_{eo})E \tag{9}$$

which, on rearrangement, gives μ_{el} in terms of experimentally measurable parameters as:

$$\mu_{el} = \mu_{eo} - L/t_R E \tag{10}$$

As shown in Fig. 4, the expression $(\mu_{el.1} - \mu_{el.2})/(\mu_{el.av} + \mu_{eo})$ was constant for a given buffer concentration, regardless of electric field strength. This was observed not only for a 0.01 M buffer in a 50-μm capillary, where Joule heating was minimal, but also for

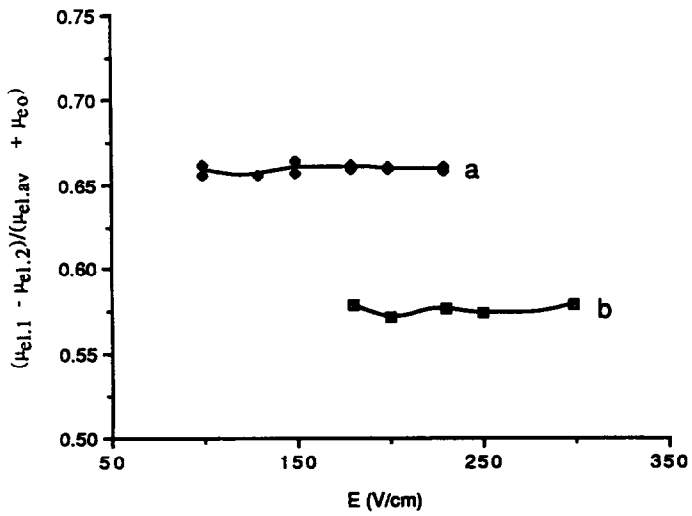

Fig. 4. Influence of buffer concentration and electric field strength (E) on the relative velocity difference $(\mu_{el.1} - \mu_{el.2})/(\mu_{el.av} + \mu_{eo})$. Conditions: a = 0.02 M buffer/100-μm capillary; b = 0.01 M buffer/50-μm capillary.

0.02 *M* Na_2HPO_4 in a 100-μm capillary where appreciable Joule heating was observed at high electric field strengths. The independence of the relative velocity difference with Joule heating is presumably attributable to the both μ_{el} and μ_{eo} having the same viscosity dependence (eqns. 5 and 6)[6].

The average values of the relative velocity difference were 0.576 and 0.661 in the 0.01 and 0.02 *M* buffers, respectively. Thus, on the basis of the relative velocity difference alone, resolution was improved in 0.02 *vs.* 0.01 *M* buffer, by a factor of 1.15. The influence of efficiency must, however, also be explored.

If it is assumed that no Joule heating occurs, as is presumably the case when 50-μm capillaries and low electric field strengths are employed, eqn. 7 may be rewritten to express resolution as a function of efficiency as:

$$R_s = f(v/2D)^{1/2} \tag{11}$$

If it can, additionally, be assumed that diffusion coefficients are the same in each buffer, resolution as a function of efficiency, in 0.02 *vs.* 0.01 *M* buffer, can be shown to be decreased by the ratio $(v_{0.02}/v_{0.01})^{1/2}$, where $v_{0.02}$ and $v_{0.01}$ are the linear velocities in 0.02 and 0.01 *M* buffer, respectively. At low electric field strengths (100 V/cm), the linear velocities of phenol were determined to be $4.96 \cdot 10^{-2}$ (0.02 *M*) and $5.96 \cdot 10^{-2}$ cm/s (0.01 *M*). Accordingly, the resolution as a function of efficiency ratio was 0.912.

Multiplication of the relative velocity difference and efficiency ratios yielded a value of 1.05. It is thus seen that the more concentrated buffer did not provide a significant improvement in resolution. Additionally, as higher electric field strengths or larger internal diameter capillaries were used, the degree of Joule heating increased to a greater extent in the 0.02 *M* buffer (Fig. 3). As shown in Fig. 5, Joule heating

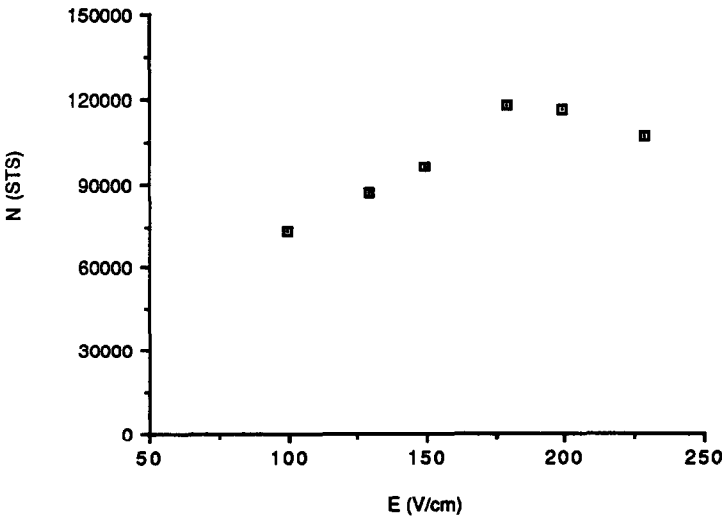

Fig. 5. Influence of electric field strength (*E*) on the efficiency (*N*) of STS. Conditions: buffer: 0.02 *M* Na_2HPO_4; capillary: 100 μm.

actually decreased efficiency at high electric field strengths, when the separation was performed in a 100-μm capillary using 0.02 M buffer. When appreciable Joule heating occurs, the resolution as a function of efficiency ratio will, consequently, be reduced with respect to the ratio calculated in the diffusion-limited case and resolution is predicted to be improved in the less concentrated buffer.

By comparing Figs. 2 and 3, it can be seen that Joule heating was much less appreciable in 50- *vs.* 100-μm capillaries. To maximize resolution, the capillary internal diameter should, therefore, be minimized regardless of the buffer concentration used.

It may, however, be undesirable to use 50-μm capillaries in applications where detectability is a problem. Defining N as:

$$N = \frac{t_R^2}{\sigma^2} \tag{12}$$

where σ^2, the total variance, is the summation of independent sources of zone dispersion; it is seen that each source of variance should be minimized. The variance contribution from sample volume, σ_S^2, may be expressed as[16]:

$$\sigma_S^2 = \frac{\tau^2}{12} \tag{13}$$

where τ, the sampling time, is related to the sample volume, S, introduced. For a cylindrical capillary of internal diameter, d_c, the relationship is:

$$\tau = \frac{S}{v\pi(d_c/2)^2} \tag{14}$$

Combination of eqns. 13 and 14 allows for σ_S^2 to be expressed as:

$$\sigma_S^2 = \frac{S^2}{12\ v^2\pi^2(d_c/2)^4} \tag{15}$$

which predicts that 16 times the sample volume may be introduced in 100- *vs.* 50-μm capillaries to yield the same σ_S^2. Thus while the zone concentration in 50-μm capillaries is four times that obtained in 100-μm capillaries (assuming equal efficiencies and migration times), concentration detection limits are predicted to be improved in the larger capillaries by virtue of the larger sample volume allowed.

The latter prediction is, additionally, influenced by the specific design of the detector employed and the experimental values of N and t_R obtained in 50- and 100-μm capillaries. However, its validity can readily be shown to be upheld. For on-capillary UV detection, generating the same noise level for 50- and 100-μm capillaries, the detection limits are improved for the larger capillary as the result of a longer pathlength (the specific improvement is governed by the detector slit width and the capillary position in the light path).

The influence of t_R and N on the detection limit may be evaluated from the peak

area (A) of an eluting analyte. The area of a Gaussian peak may be described by the expression[16]:

$$A = 2.51\sigma h \tag{16}$$

where h is the height at the peak apex. Eqn. 16 may be combined with eqn. 12 and rearranged to yield:

$$h = \frac{AN^{1/2}}{2.51t_R} \tag{17}$$

Thus, for the same peak area, a larger peak height (signal) will result in the higher efficiency case. In practice however, the efficiency enhancement observed in 50- *vs.* 100-μm capillaries is minimal (as will be shown) and as a result does not offset the sample-size advantage obtained in the larger capillaries. Furthermore, as illustrated in Figs. 2 and 3, the decrease in efficiency resulting from Joule heating is countered by a smaller t_R value by virtue of increased electroosmotic flow.

From the foregoing, it may be concluded that improved concentration detection limits are obtained in 100-μm capillaries. This improvement is, however, accompanied by a decrease in efficiency which may be required to effect the separation. To achieve a compromise between detectability and resolution, it is therefore desirable to find a method of reducing Joule heating. The theoretical work of Knox[12] has predicted that the Joule heat generated may be dissipated by forced convection. This prediction is supported by the experimental work of Boček *et al.*[13] and Nelson *et al.*[17] and by these results, as shown in Fig. 6. When forced air convection at 3.5 l/min was applied to

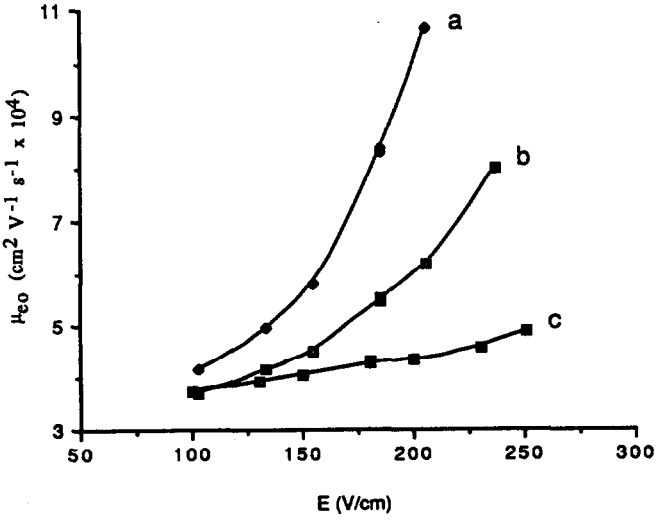

Fig. 6. Influence of forced convection on the coefficient of electroosmotic flow (μ_{eo}) in 0.05 M Na_2HPO_4[13]. Capillaries: a = 100 μm; b = 100 μm with convection; c = 50 μnm.

50 cm of the 100-μm capillary (Fig. 1), μ_{eo} as a function of electric field strength was reduced in comparison to the same capillary in the absence of forced convection.

As a result of the reduction in Joule heating, efficiency was improved. Initial results showed the efficiency for phenol in 0.05 M Na_2HPO_4 at 18 kV/m to increase from 112 000 to 117 000 theoretical plates when forced convection was employed[18]. In these initial studies however, high concentrations of phenol were employed and consequently an additional source of band broadening was incurred[4,13,19]. When the analyses were repeated at 22.7 kV/m and a $2 \cdot 10^{-4}$ M phenol solution used as the sample, efficiencies were improved from 191 000 $\pm$ 3.1% relative standard deviation (R.S.D.) to 226 000 $\pm$ 3.3% R.S.D. theoretical plates (based on 4 determinations). For comparison, the efficiency obtained in 50-μm capillaries, under similar operating conditions, was approximately 264 000 theoretical plates.

As can be seen from Fig. 6, more effective forced convection is required to reduce μ_{eo} to approach the level observed in 50-μm capillaries. Work to achieve this via changes in the air flow-rate, changes in the chemical nature of the dissipant and convection along the entire length of the capillary, is currently ongoing.

ACKNOWLEDGEMENTS

H.T.R. wishes to thank the San Francisco Bay Area Chromatography Colloquium and the Barnett Institute of Northeastern University for a travel grant which made the presentation of this paper at HPCE '90 possible.

REFERENCES

1 J. C. Giddings, *Sep. Sci.*, 4 (1969) 181.
2 J. W. Jorgenson and K. D. Lukacs, *Anal. Chem.*, 53 (1981) 1298.
3 A. W. Adamson, *Physical Chemistry of Surfaces*, Wiley-Interscience, New York, 4th ed., 1981, Ch. 5.
4 K. D. Lukacs and J. W. Jorgenson, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 407.
5 K. D. Altria and C. F. Simpson, *Chromatographia*, 24 (1987) 527.
6 S. Hjerten, *J. Chromatogr.*, 347 (1985) 191.
7 G. J. M. Bruin, J. P. Chang, R. H. Kuhlman, K. Zegers, J. C. Kraak and H. Poppe, *J. Chromatogr.*, 471 (1989) 429.
8 S. Fujiwara and S. Honda, *Anal. Chem.*, 59 (1987) 487.
9 K. D. Altria and C. F. Simpson, *Anal. Proc.*, 23 (1986) 453.
10 M. Martin, G. Guiochon, Y. Wahlbroehl and J. W. Jorgenson, *Anal. Chem.*, 57 (1985) 559.
11 E. Gruska, R. M. McCormick and J. J. Kirkland, *Anal. Chem.*, 61 (1989) 241.
12 J. H. Knox, *Chromatographia*, 26 (1988) 329.
13 F. Foret, M. Deml and P. Boček, *J. Chromatogr.*, 452 (1988) 601.
14 H. H. Lauer and D. McManigill, *Trends Anal. Chem.*, 5 (1986) 11.
15 K. D. Lukacs, *Thesis*, University of North Carolina, Chapel Hill, NC, 1983.
16 J. C. Sternberg, *Adv. Chromatogr.*, 2 (1966) 205.
17 R. J. Nelson, A. Paulus, A. S. Cohen, A. Guttman and B. L. Karger, *J. Chromatogr.*, 480 (1989) 111.
18 H. T. Rasmussen and H. M. McNair, presented at the *2nd International Symposium on High Performance Capillary Electrophoresis, San Francisco, CA, 1990*, paper P-122.
19 F. E. P. Mikkers, F. M. Everaerts and Th. P. E. M. Verheggen, *J. Chromatogr.*, 169 (1979) 1.