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Journal of Chromatography A, 849 (1999) 563–573

JOURNAL OF CHROMATOGRAPHY A

Chemometric approach to the treatment of benzodiazepine separation and peak broadening in capillary electrophoresis

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Received 18 November 1998; received in revised form 22 February 1999; accepted 28 April 1999

Abstract

A chemometric methodology was used to study capillary efficiency and the separation of ten benzodiazepines in capillary electrophoresis. The resolution between two adjacent peaks on the electropherogram was estimated and the overall quality of the separation was assessed by means of a new response function. The nature (methanol or acetonitrile) and proportion of the organic modifier both in the background electrolyte and the sample buffer and the injection time were considered. The results predicted that if the sample had a lower dielectric constant than the background electrolyte buffer then a much larger injection volume could be used. The computer optimization routine was experimentally validated and the result demonstrated that the fastest electrophoretic reparation was obtained with acetonitrile (7 min instead of 9 min with methanol).

Keywords: Chemometrics; Peak shape; Background electrolyte composition; Optimization; Benzodiazepines

1. Introduction

The vast majority of capillary electrophoresis (CE) separations have been performed using aqueous buffers as background electrolytes (BGEs). The addition of organic solvents to the BGE has been studied in capillary zone electrophoresis (CZE) [1–3] and micellar electrokinetic chromatography [4,5], but the concentration of modifiers has rarely exceeded 40% [6,7]. Several groups investigated the utility of organic solvent modifiers in CZE [8–10]. Creenaway et al. reported significant improvements in the resolution of hydrophobic compounds when a mixture of deuterium oxide and deuterated methanol were used instead of methanol and water [11]. Kitagawa and Tsuda reported that the electroosmotic flow velocity was almost constant with 30–90% (v/v) in the elution solvent [12]. Other groups investigated the use of a non aqueous separation medium. Janson and Roeraade [13] studied the separation of compounds with poor water solubility in an *N*-methylformamide (NMF) medium. Miller et al. [14] recently reported the separation of polycyclic-aromatic-hydrocarbon's (pAHs) in a purely non aqueous-medium (acetonitrile) with the tropylium and 2,4,6-triphenylpyrylium ions serving as charge-transfer agents. They reported that the migration of the pAHs was generally by size, with the largest migrating the fastest through the capillary. Other reports of non aqueous media for CE have included the separation of aromatic and aliphatic acids in tris-acetate–methanol solvent [15], the analysis of long chain surfactants [16] and the analysis of

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00596-8

porphyrins in organic media with various additives [17,18]. Finally Wright et al. [19] evaluated acetonitrile, formamide, methanol, dimethylformamide (DMF), and dimethyl sulfoxide (DMSO), without the addition of supporting electrolytes as solvents for non aqueous CE separation. Several multivariate statistical approaches for the simultaneous study of multiple CE operating variables have been investigated [20–22]. A Plackett Burman [21] experimental design was utilized to optimize heptakis (2,3,6-tri-*O*-methyl)-β-cyclodextrin (TMCD), methanol, buffer pH, ionic strength, and hydrodynamic injection time for resolution and analysis time. Experimental design optimization methods have been shown to be particularly useful for the rapid development of the optimum buffer composition and separation conditions. Varesio [23] explored a central composite experimental design to evaluate five operational variables for the resolution of amphetamine in CE. This paper discusses, using experimental data, improvements in peak efficiency when the injection time varied and the organic modifier is changed from methanol to acetonitrile in both the sample buffer and the BGE. A new optimization process that obtained an efficient separation in a minimum analysis time was also proposed.

2. Methods

2.1. Plate height

In CE, as in chromatography, the various contributions to overall peak broadening can be described by their contributions to the overall plate height. The plate height is calculated by:

$$\mathrm{H} = \frac{\sigma^2}{L_\mathrm{d}} = \frac{L_\mathrm{d}}{16(t_\mathrm{M}/W_\mathrm{B})^2} \tag{1}$$

where L_d is the capillary length to the detector, σ^2 the peak dispersion, t_M is the migration time, and W_B the peak width at the baseline.

2.2. Model for zone variance

In this work, two principal sources of broadening were considered: diffusion (σ^2_dif) and injection volumes (σ^2_inj).The variance resulting from diffusion by time unity (σ^2_dif/t) is given by the Janson and Roeraade equation [13]:

$$\frac{\sigma^2_\mathrm{dif}}{t} \propto \frac{k_\mathrm{B} T \eta}{rE} \cdot \frac{1}{(2\xi_\mathrm{s} - 3\xi_\mathrm{wall})^2 (\epsilon_o \epsilon_r)^2} \tag{2}$$

where r is the Stockes radius of the solute, k_B the Boltzmann constant, T the temperature, η the solvent viscosity, ξ_s and ξ_wall are the zeta potentials of the analyte and the capillary wall, respectively, ϵ_o the permittivity of vacuum, ϵ_r the relative dielectric constant and E the electric field strength. The product $\epsilon_o \epsilon_r$ is equal to ϵ which is the permittivity. The contribution to variance that caused a finite injection volume is approximated by the expression given by Sternberg [24]:

$$\sigma^2_\mathrm{inj} = \frac{l^2_\mathrm{inj}}{12} \tag{3}$$

where l_inj is the length of the injection zone. In hydrodynamic injection, the sample is introduced by establishing a pressure gradient along the capillary for a brief period of time. Under the condition of laminar flow, the length of the injection zone is determined by means of the Hagen–Poiseuille equation [25]:

$$l_\mathrm{inj} = (\Delta p \cdot r^2 / 8 \eta L_\mathrm{t}) t_\mathrm{inj} \tag{4}$$

where t_inj is the injection time, L_t the total capillary length, and Δp is the pressure drop.

2.3. Dielectric constant variation between BGE and sample buffer

In the volume fraction range used for solvents, the volume change of mixing was negligible [26,27] and was not considered in the following equations. Thus, If d_OM and $d_{\mathrm{H_2O}}$ are the molar density of the organic modifier (OM) and water, the molar fractions of OM, x_OM, and water, $x_{\mathrm{H_2O}}$ can be given by the following equations [26,27]:

$$x_\mathrm{OM} = d_\mathrm{OM}(1-\Phi)/(d_{\mathrm{H_2O}}\Phi + d_\mathrm{OM}(1-\Phi) \tag{5}$$

$$x_{\mathrm{H_2O}} = d_{\mathrm{H_2O}}\Phi/(d_{\mathrm{H_2O}}\Phi + d_\mathrm{OM}(1-\Phi) \tag{6}$$

The dielectric constant of the OM–water mixture was given by [27]:

$$\epsilon = x_{H_2O}\epsilon^{\circ}_{H_2O} + x_{OM}\epsilon^{\circ}_{OM} \quad (7)$$

where $\epsilon^{\circ}_{H_2O}$ and ϵ°_{OM} were the dielectric constants of pure water and OM, respectively. When the fraction of OM in the sample buffer or in the BGE changed the variation, $\Delta\epsilon$, of the dielectric constant between these two solutions was given by:

$$\Delta\epsilon = \epsilon^{\circ}_{H_2O}\,\Delta x_{H_2O} + \epsilon^{\circ}_{OM}\,\Delta x_{OM} \quad (8)$$

where

$$\Delta x_{H_2O} = x_{H_2O,c} - x_{H_2O,s} \quad (9)$$

$$\Delta x_{OM} = x_{OM,c} - x_{OM,s} \quad (10)$$

where the substricts *c* and *s* refer to BGE and sample buffer respectively. In the following, $x_{H_2O,c}$, and thus $x_{OM,c}$, were maintained constant and $x_{H_2O,s}$ and $x_{OM,s}$ were chosen so that the sample buffer had a lower dielectric constant than the BGE.

2.4. Resolution

For a quantitative description of a mutual separation of two adjacent analytes on an electropherogram, the dimensionless resolution R_s was used [28,29]:

$$R_s = \frac{1}{4}\frac{\Delta\mu}{\bar{\mu}}\sqrt{L_d/H} \quad (11)$$

where $\Delta\mu$ is the difference in electrophoretic mobility between two solutes, $\bar{\mu}$ the average electrophoretic mobility of the two species.

2.5. Optimization strategy

The quality of the entire separation of the ten solutes was assessed by means of a new response function ξ defined as:

$$\xi = \mathrm{Min}(R_S) \quad \text{if Min}\,(R_S) \le R_l \quad (12)$$

$$\xi = R_l + 1/t_a \quad \text{if not}$$

where $\mathrm{Min}(R_S)$ is the resolution for the worst separated pair of peaks on the electropherogram. R_l was called the limit resolution and is the minimum value of the resolution accepted. In our application, R_l was 0.8. Therefore, if the resolution for the worst separated pair of peaks on the electropherogram was lower than the chosen limit resolution, then the ξ function would be equal to the resolution. If not, separation conditions were obtained and then the analysis time t_a inverted in the form $1/t_a$. Thus, the ξ function was maximal when both efficient separation conditions and a minimal time were obtained. The analysis time t_a was given by the migration time of the last compound on the electropherogram. t_a was given by the well known equation:

$$t_a = \frac{L_t L_d}{V}\cdot\frac{1}{\mu} \quad (13)$$

where V is the applied voltage and μ the electrophoretic mobility of the last compound.

2.6. Chemometric methodology

The chemometric approach is based on factorial designs. Two level factorial designs give a fitting of a first order (linear) model to the data [30]. If the effects of each of the three factors do not vary linearly, a design which requires 13 experiments to detect curvature in the response can be used. Thus, the Box and Benhken design [31–33] was developed specifically to enable a response surface to be fitted to the data, as it provides sufficient information for the fitting of a quadratic model to a data set. These models are amenable to regression analysis. For three factors this takes the form of:

$$y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 + a_{12}x_1x_{2+}a_{13}x_1x_3 + a_{23}x_2x_3 \quad (14)$$

where y is the response or dependent variable, and x_1, x_2, x_3 are the logarithms of respectively the percentage of OM (θ%) in the OM–buffer mixture (BGE), the percentage of the OM (β%) in the OM–buffer mixture of the sample and injection time t_{inj} (s).

2.7. Simplex optimization

To optimize the mathematical model (y) given by the experimental design, a simplex method was used. The y value was calculated for m sets of starting

conditions, where m was given by the number of factors to be optimized plus 1. Therefore, in this case m was four. The point corresponding to the lowest value of y was then reflected in relation to the surface defined by the three other points to give a fifth set of starting conditions. Once again, the point with the lowest y was reflected and the process repeated sequentially, until the same values for θ, β, and t_{inj} continued to be selected.

3. Experimental section

3.1. Apparatus

CE separations were carried out using an automated CE apparatus (Beckman, Pace-550, Paris, France). The capillary used was 57 cm (50 cm to the detector)×75 μm I.D. The following conditions were: applied voltage 30 kV; capillary thermostated at 25°C unless otherwise specified; UV detection at 230 nm; 2.s. pressure injection of benzodiazepine solution dissolved in the BGE.

3.2. Solvents and samples

Water was obtained from an Elgestat option I water purification system (Odil, Talant, France) fitted with a reverse osmosis cartridge. The BGE buffer and sample buffer consisted of a mixture of acetonitrile (ACN) and phosphate buffer composed of 0.05 *M* diammonium hydrogenphosphate and 0.05 *M* ammonium dihydrogenphosphate. The phosphate buffer pH was adjusted to 2.3 with phosphoric acid. The range of the ACN fraction (v/v) was 0.06–0.66 in the BGE and 0.1 –0.94 in the sample buffer. Oxazepam (1), tofisopam (2), diazepam (3), chlorazepate dipotassic (4), chlordiazepoxide (5), flunitrazepam (6), clobazam (7), bromazepam (8), nitrazepam (9), and lorazepam (10) were obtained from Hoffman Laroche (Basel, Switzerland). Each benzodiazepine of a concentration of 0.9 m*M* or a mixture of these when the ten compounds' peaks were well resolved was injected and the migration times were measured.

4. Results and discussion

The experimental H were determined from the electropherograms using Eq. (1). All experiments were repeated three times. The coefficient of variation of the H values was less than 2% in most cases, indicating a high reproductibility and good stability for the electrophoretic system. Using the experimental design, the inverse of the height to a theoretical plate ($1/H$) was modelled by Eq. (14). Eqs. (2) and (3) were not used to calculate these theoretical values of H. From the full regression model (Eq. (14)) a Student t-test was used to provide the basis for the decision as to whether or not the model coefficients were significant. Results of the Student t-test showed that no variables can be excluded from the model. This generated model was assessed statistically using a Fischer–Schnedecor test (F-test) and a coefficient of multiple determination R^2. For the ten solutes, for which H was modelled, these criteria were at least equal to 310 and 0.979 for OM=MeOH or OM=ACN. These values demonstrated an excellent validity for the model. For example, experimental and calculated values of H for solute diazepam and OM=ACN are summarized in Table 1. These values were chosen in the parameter space, and not in the 13 experiments given by the experimental design to show that the H-model was within the range.

4.1. Sources of extraseparation broadening

There are numerous sources of extraseparation broadening [24]. One of these occurs with the practice of on-column detection in CE. On-column detectors monitor a finite length of the capillary. When the length of the detection zone approaches the width of the sample zone, the resultant apparent peak is broadened. The cartridges in the CE system were supplied with aperture widths of 100, 200, and 800 μm, i.e. the illuminated length of the capillary can range from 100 to 800 μm. For $\theta=66\%$, $\beta=10\%$, and $t_{inj}=2$ s, the 100 μm detector width yielded $H=3.20$ μm, whereas the 800 μm width reduced the efficiency to $H=3.35$ μm. The relative difference between these two values is 4.5%. For the other shortest injection times, the relative difference was always $\leq 5\%$. However, for these same values,

Table 1
Calculated and experimental H values (H_{cal} and H_{exp} for different values of θ (%), β (%) and t_{inj} (s) with ACN as organic modifier

θ (%)	β (%)	t_{inj} (s)	$H_{ca\ l}$(μm)	H_{exp} (μm)
6	10	2	4.24	4.14
10	16	3	4.07	4.11
14	22	4	5.10	4.99
18	28	5	7.10	7.21
22	34	6	7.80	7.95
26	40	7	10.10	10.16
30	46	8	9.82	10.00
34	52	9	17.11	14.22
38	58	10	13.13	13.31
42	64	2	5.13	5.18
46	70	3	7.12	7.20
50	76	4	8.00	7.95
54	82	5	7.82	8.00
58	88	6	5.86	5.97
62	94	7	6.70	6.79
66	10	8	7.10	7.14
6	16	9	7.22	7.16
10	22	10	8.00	8.15
14	28	2	4.83	4.88
18	34	3	5.22	5.17
22	40	4	7.00	6.89
26	46	5	8.10	8.18
30	52	6	7.80	7.95
34	58	7	8.00	8.21
38	64	8	9.00	9.16
42	70	9	10.10	10.22
46	76	10	9.14	9.10
50	82	2	4.12	4.11
58	88	3	3.23	3.26
62	94	4	4.22	4.28

$\theta = 66\%$, $\beta = 10\%$ when the injection time increased from 2 s to 5 s, the efficiency decreased: 18%, 19%, and 20% for an aperture width of 100, 200 and 800 μm. Therefore, injection broadening is a much greater potential source of extraseparation broadening than the detection width. Thus, the discussion below focuses solely on our experiments with broadening caused by injection and the composition of both the background electrolyte and the sample buffer.

4.2. Variation of H versus the BGE buffer composition

Eq. (14) showed that for a constant injection time (or sample buffer composition) the variation of H versus the OM percentage presented a maximum (minimum efficiency). The viscosity had a maximum at approximatively $\theta \sim 25\%$ for methanol and at $\theta \sim 45\%$ for acetonitrile [34]. Beyond this maximum, it is possible that the better solubility of the weak polar solute reduced its capillary wall binding. The factor peak band broadening due to the solute adsorption on the capillary wall decreased. The optimum value of the plate height was determined using the simplex optimization method. Forty iterative processes were performed by the computer for solute diazepam and ACN (35 for MeOH). The results, for ACN, are set out in Table 2. The optimum values for methanol were $\theta = 64.40(\%)$, $\beta = 93.20(\%)$, $t_{inj} = 2.10$ s, those for acetonitrile, were, $\theta = 58.42(\%)$; $\beta = 90.80(\%)$; $t_{inj} = 2.20$ s. The corresponding calculated H values were 3.90 μm for MeOH and 2.10 μm for ACN. The experimental values of H for these conditions were 3.82 μm and 2.14 μm. In the optimum conditions, capillary efficiency was approximatively two times less efficient with MeOH than with ACN. Table 3 systematically sets out the experimental and theoretical plate heights in a large variation of percentages of OM in the BGE for solute diazepam. The values were found to be markedly dependent on the organic component in the buffer. For MeOH, the plate height H was ≤ 3.90 μm corresponding to a plate number $N \geq 192\,000$ and for ACN, $H \leq 2.10$ μm corresponding to a plate number $N \geq 341\,000$. From the data in Table 3, it can be seen that for a given θ value, for ACN, the capillary was two or four times more efficient than with MeOH. For example, if $\theta = 42\%$, $\beta = 10\%$ and $t = 2$ s, the experimental H value was 6.80 μm for OM = ACN and 12.01 μm for OM = MeOH. A new equation was developed relating H to the nature of OM and its composition in the BGE. For a given couple, the sample buffer composition/injection time and using Eq. (14), the following analytical equation links $1/H$ to the fraction of OM in the BGE:

$$1/H_{OM} = \alpha_1 + \alpha_2 \ln \theta + \alpha_3 (\ln \theta)^2 \quad (15)$$

where α_1, α_2, α_3 were constant and H_{OM} was the plate height in the OM–BGE mixture. Thus, the following can be written:

$$1/H_{ACN} - 1/H_{MeOH} = V(\theta) \quad (16)$$

$$V(\theta) = \Delta\alpha_1 + \Delta\alpha_2 \ln \theta + \Delta\alpha_3 (\ln \theta)^2 \quad (17)$$

Table 2
Result of the simplex process for the optimization of H (μm) with ACN as the organic modifier

Experiment no.	θ (%)	β (%)	t_{inj} (s)	H (μm)
1	6.00	10.00	10.00	11.23
2	6.10	10.40	9.70	11.47
3	6.75	11.20	10.00	12.93
4	7.10	12.00	8.82	9.10
5	8.44	20.00	7.75	7.14
6	12.44	25.10	5.42	7.00
7	7.10	20.10	5.40	6.42
8	25.40	25.10	3.20	8.10
9	26.40	35.10	2.30	7.10
10	26.70	40.10	3.40	8.30
11	32.80	40.14	5.15	9.00
12	33.85	38.13	6.20	9.10
13	25.70	45.16	3.10	8.12
14	36.42	50.12	4.10	9.10
15	42.34	50.42	3.20	8.40
16	45.00	54.40	2.10	6.40
17	48.32	55.10	4.40	8.10
18	49.44	60.22	5.20	8.12
19	52.30	60.40	6.20	10.11
20	54.40	61.50	3.40	7.42
21	57.40	65.00	7.30	8.44
22	58.40	70.40	6.50	6.23
23	48.22	71.40	5.40	7.11
24	47.32	75.30	4.20	6.88
25	53.33	77.40	3.20	4.44
26	57.20	75.40	3.40	4.20
27	55.20	78.40	3.00	4.48
28	60.20	81.30	3.12	3.21
29	62.34	82.40	2.80	2.90
30	65.00	84.30	2.10	2.44
31	58.20	85.30	2.25	2.10
32	58.42	90.30	2.20	2.15
33	58.42	89.90	2.22	2.11
34	58.48	90.40	2.20	2.10
35	58.42	90.50	2.20	2.12
36	58.42	90.80	2.19	2.14
37	58.45	90.78	2.18	2.10
38	58.42	90.85	2.17	2.12
39	58.42	90.80	2.20	2.10
40	58.42	90.80	2.20	2.10

In all cases, the correlation coefficients for the fits were always more than or equal to 0.982. In each case, the differences $\Delta\alpha_1$, $\Delta\alpha_2$, and $\Delta\alpha_3$ were of the same sign and similar range. In the interval (6–66%), the polynomial $V(\theta)$ was always greater than zero indicating that $H_{ACN} \leq H_{MeOH}$. This conclusion can be supported by the fact that solvents with high ϵ^2/η values provide high efficiency [13].

Table 3
Calculated and experimental H (μm) values for different percentages of organic modifier OM in the BGE buffer, $\beta = 10\%$ and $t_{inj} = 2$ s

θ(%)	Acetonitrile		Methanol	
	H_{cal} (μm)	H_{exp} (μm)	H_{cal} (μm)	H_{exp} (μm)
6	4.24	4.14	8.90	8.75
18	6.22	6.20	9.60	9.38
30	7.10	7.08	10.20	10.78
42	6.90	6.80	12.79	12.01
54	5.14	5.10	13.00	13.14
66	3.22	3.20	11.34	11.31

4.3. Variation of H versus Δε and the injection time

As indicated above, all the parameters have a significant effect on the plate height. The retention of the second order terms in the reduced model x_2^2 and x_3^2 demonstrated that both the sample buffer composition and injection time influenced the degree of curvature of the response surface, H vs. (t_{inj}, $\Delta\epsilon$). As can be seen from Figs. 1 and 2 the following conclusion can be drawn:

For a constant $\Delta\epsilon$ value (Fig. 1) when t_{inj} was less or equal to a critical value $t_{c,inj}$, the plate height values showed weak or nil variations. Above $t_{c,inj}$ the plate height largely increased with t_{inj}.

As $\Delta\epsilon$ increased $t_{c,inj}$ increased (Fig. 2). A linear relationship was found for all solutes and for both ACN and MeOH. For solute diazepam and OM = ACN the following was obtained:

$$t_{c,inj} = 2.10 + 0.35\Delta\epsilon \quad (18)$$

The coefficients of determination r^2 for all fits were always more than or equal to 0.979. The typical standard deviation of slope and intercept were respectively 0.004 and 0.05. The interpretation of these observations is straightforward. In non aqueous media, currents are lower than aqueous buffer of the same ionic strength [35]. Thus, as $\Delta\epsilon$ increased the conductivity in the sample buffer was always lower than in the BGE and decreased [35]. The electric field in the low conductivity of the sample buffer was higher than that in the BGE. Thus, a stacking phenomenon occurred. Solutes within the sample rapidly migrated to the interface between the sample

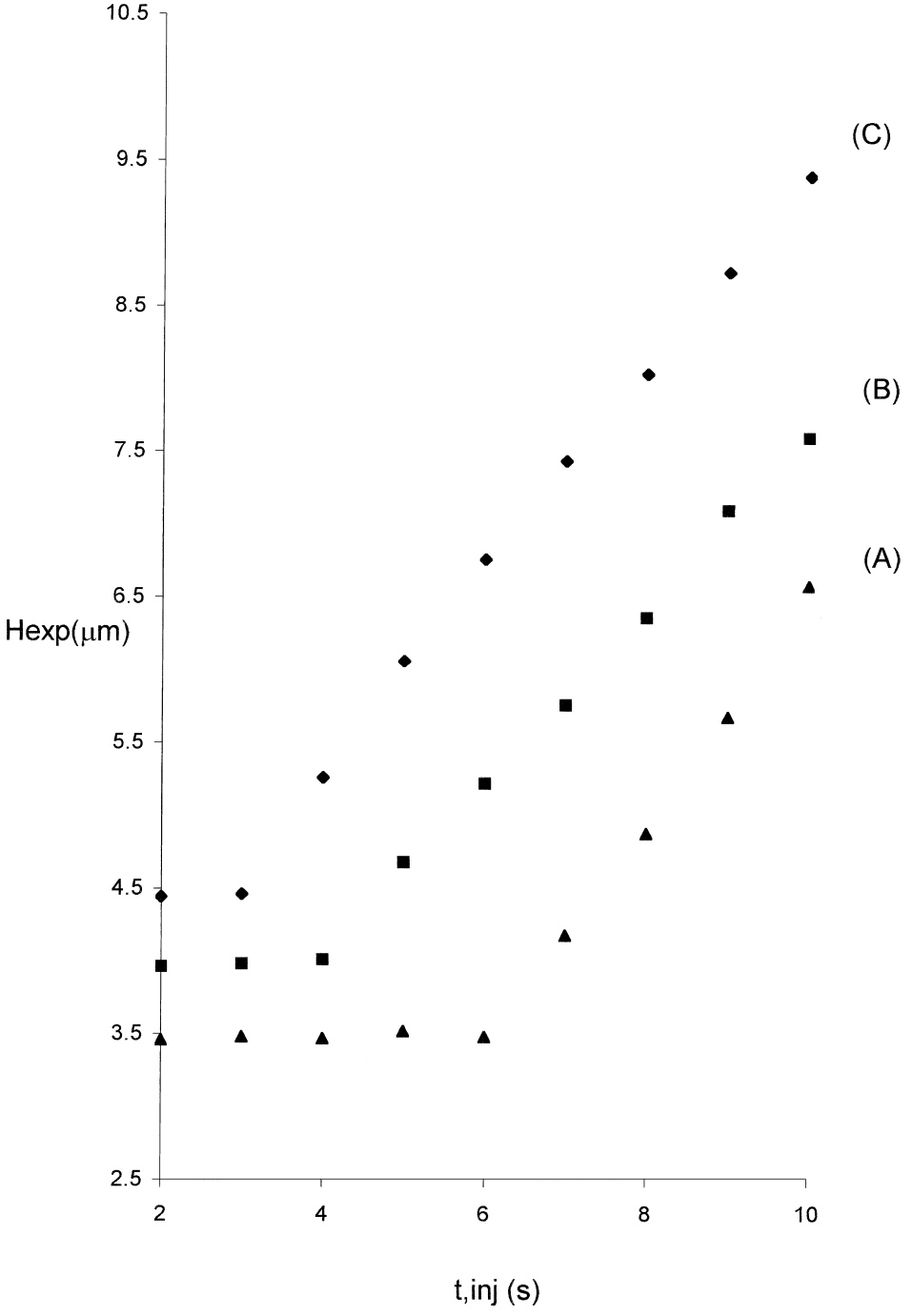

Fig. 1. Plot of H_{exp} (μm) versus t_{inj} for: (A) $\Delta\epsilon = 11.9$, (B) $\Delta\epsilon = 5.9$, (C) $\Delta\epsilon = 0.8$. OM was ACN.

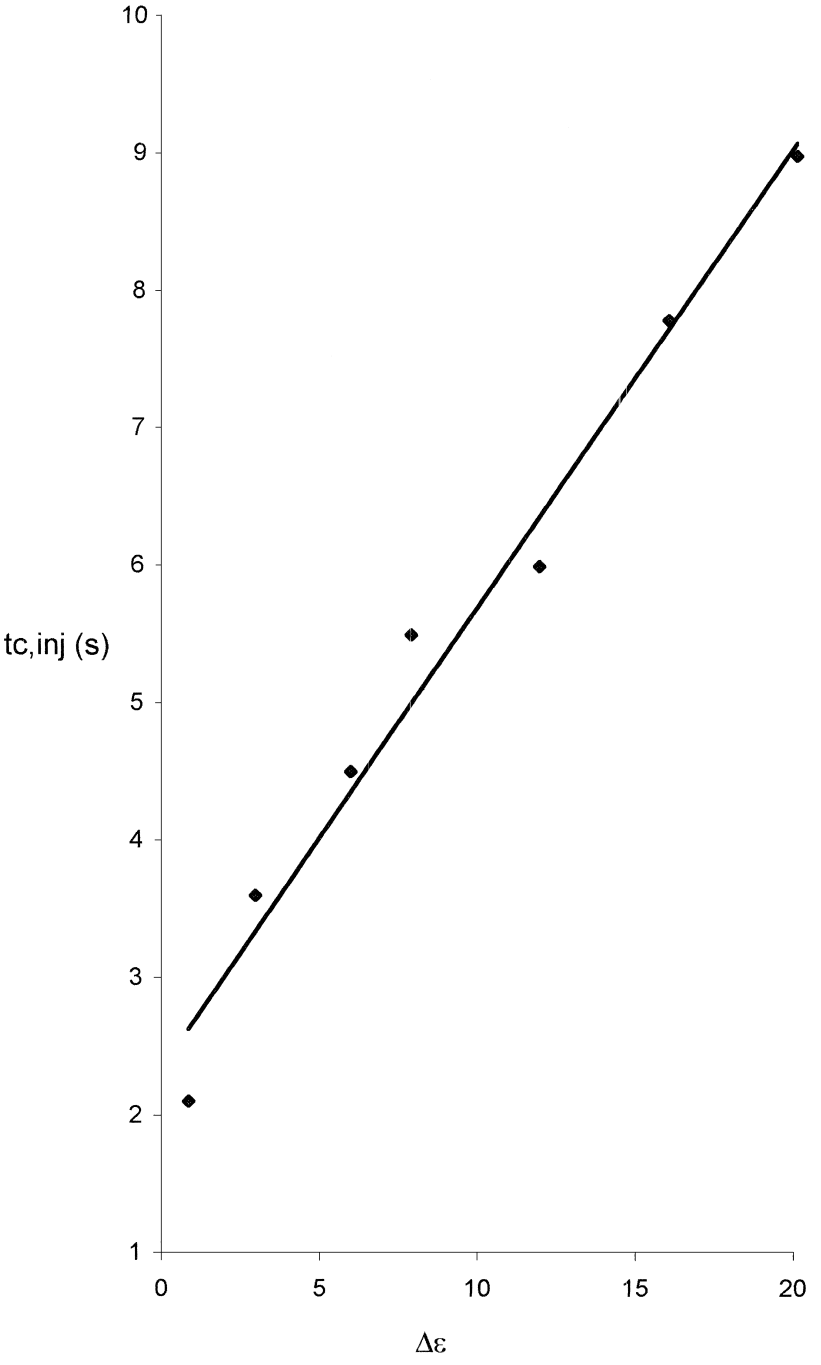

Fig. 2. Plot of $t_{c,inj}$ (s) versus $\Delta\epsilon$. OM was ACN.

and BGE. Upon reaching the interface, the local field strength decreased, which caused the solute to slow down and stack in a narrow band. Therefore, as $\Delta\epsilon$ increases longer injection time can be used and at the same time this preserves efficiency. Nevertheless, the rapid increase in the length of the injection zone with an increase in the injection time produces an increase in the peak broadening which necessitated a higher plate number. Thus, above the critical injection time value, $t_{c,inj}$, this second effect supplanted the stacking phenomenon and the column plate height increased. These results demonstrate the importance of controlling the injection time and the composition of the sample buffer to improve the quality of an electrophoretic separation.

4.4. Separation optimization

The coefficient of variations of the electrophonetic mobilities μ of all the solutes were less than 2%. Using the experimental design, the μ logarithm for each of the ten solutes were modelled by Eq. (14). All the correlation coefficients were higher than 0.992. The Student *t*-test confirmed that for each solute the μ value was independent of the injection conditions, i.e. t_{inj}. The analysis time t_a (Eq. (13)) was given by the mobility of the last compound on the electropherogram. Computer simulations [36–42] have begun to play an increasing role in optimization separations. The utility of the ξ method is that it takes into account the analysis time t_a and the simultaneous variations in column efficiency with the three factors, BGE composition, sample buffer composition, and injection time. The experimental design reduced the number of experiments to be carried out. Therefore, knowing the variation of u, H, and t_a with the three factors, the ξ values (Eq. (12)) can be calculated for different values of the three factors. ξ reached its maximum for $\theta = 63\%$, $\beta = 85\%$, $t_{inj} = 4.2$ s for ACN and for $\theta = 64\%$, $\beta = 83\%$, $t_{inj} = 3.6$ s for MeOH. For example, the results of the simplex process using ACN, is given in Table 4. The corresponding electropherograms are given in Fig. 3. With ACN, the ten benzodiazepines separated better with an analysis time equal to 7 min instead of 9 min with MeOH.

Table 4
Result of the simplex process for the optimization of the ξ function with ACN as the organic modifier

Experiment no.	θ (%)	β (%)	t_{inj} (s)	ξ
1	30.0	10.0	5.0	0.0223
2	35.0	20.0	6.0	0.0236
3	40.6	25.4	4.0	0.0312
4	45.8	30.4	4.5	0.0313
5	30.8	40.2	5.5	0.0414
6	44.5	45.6	7.0	0.0515
7	56.8	48.6	6.5	0.0714
8	57.8	47.2	5.5	0.1120
9	48.2	50.2	3.2	0.0900
10	48.7	51.3	3.2	0.0910
11	51.4	52.3	4.4	0.1131
12	55.3	58.5	4.8	0.2148
13	57.8	57.2	6.5	0.3168
14	54.2	56.2	7.5	0.3172
15	55.8	59.3	8.0	0.2183
16	54.3	60.2	8.5	0.2231
17	48.8	65.2	9.5	0.1121
18	50.2	70.3	10.5	0.0072
19	54.8	75.3	7.5	0.2211
20	56.8	80.2	6.5	0.3481
21	60.3	80.7	5.0	0.6142
22	60.8	81.3	5.5	0.6232
23	64.5	87.4	4.5	0.7211
24	61.8	85.4	4.4	0.7223
25	63.2	70.4	4.5	0.6999
26	63.3	84.9	5.0	0.8220
27	63.2	85.0	4.5	0.8730
28	63.3	85.0	4.2	0.8734
29	63.3	85.1	4.2	0.8741
30	63.4	85.3	4.2	0.8748

5. Conclusion

The use of ACN instead of MeOH improved capillary efficiency. The results are corroborated by simple new equations relating the capillary efficiency to both the nature of the organic modifier and its percentage in the electrophoretic buffer. The results obtained demonstrated the need to control the composition of the non aqueous sample buffer to be able to use a much larger injection time and at the same time preserves capillary efficiency. As well, the separation of the ten benzodiazepines and the analysis time were both optimized with a new response function developed in our laboratory. The results

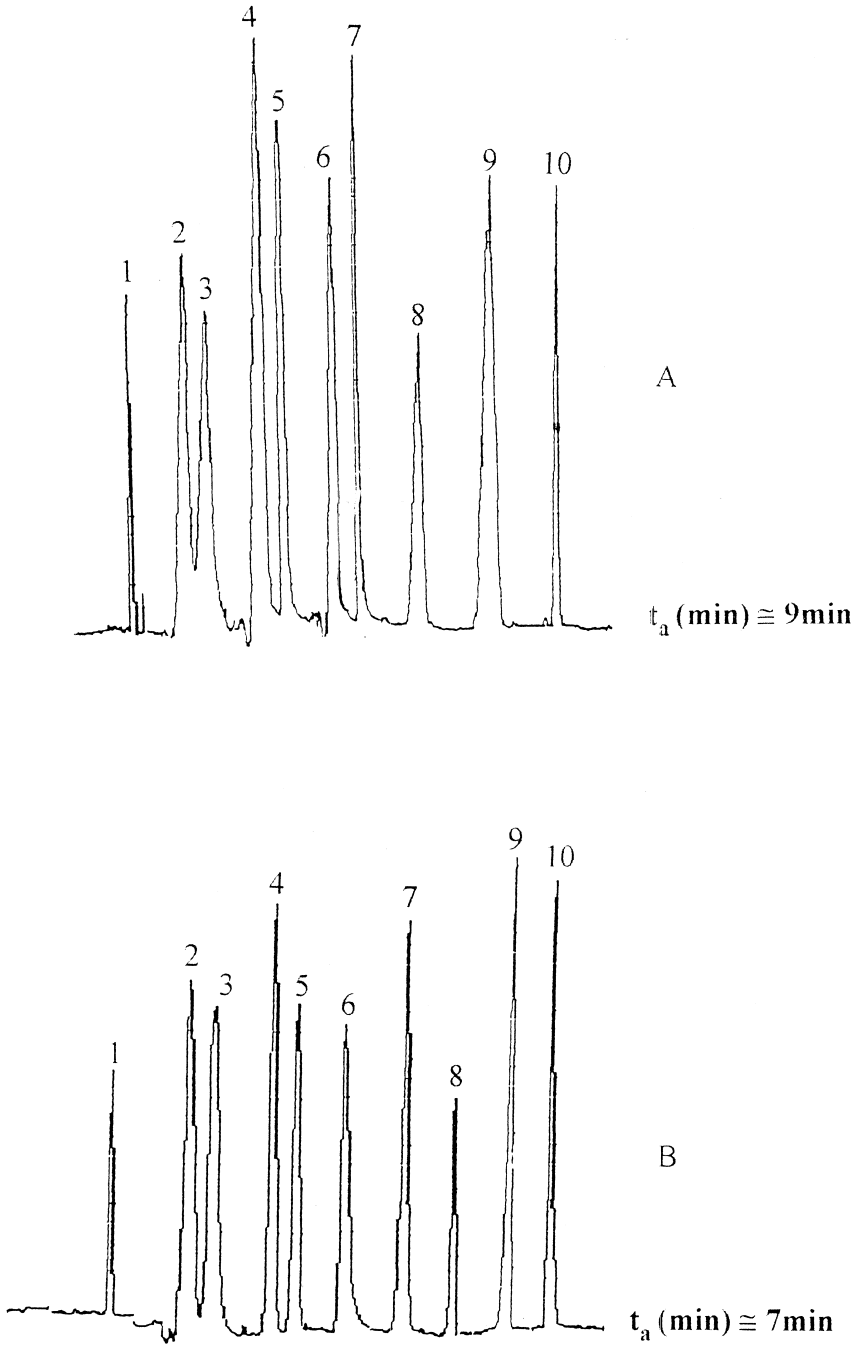

Fig. 3. Benzodiazepine electropherogram in the optimum conditions: (A) OM=MeOH, θ=64%, β=83%, t_{inj}=3.6 s; (B) OM=ACN, θ=63%, β=85%, t_{inj}=4.2 s. Number above peaks refers to the ten compounds: see solvents and samples.

showed that the analysis time was reduced when ACN was used instead of MeOH.

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