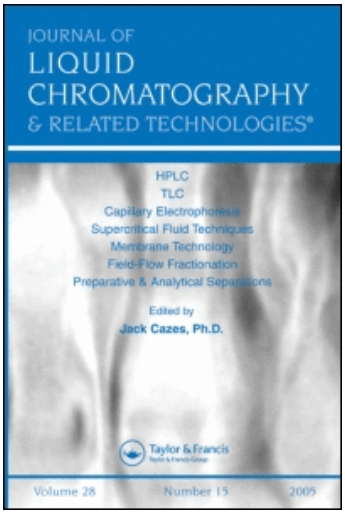


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

OPERATING PARAMETER EFFECTS IN CAPILLARY ZONE ELECTROPHORESIS (CZE): ANALYSIS OF PENTAMIDINE, HYDROXYSTILBAMIDINE, PROPAMIDINE, DAPI, AND STILBAMIDINE IN BODY FLUIDS

B. Rabanal[a]; E. de Paz[a]; N. Walser[a]; A. Negro[a]
[a] Universidad de León, León, Spain

Online publication date: 22 January 2001

To cite this Article Rabanal, B. , de Paz, E. , Walser, N. and Negro, A.(2001) 'OPERATING PARAMETER EFFECTS IN CAPILLARY ZONE ELECTROPHORESIS (CZE): ANALYSIS OF PENTAMIDINE, HYDROXYSTILBAMIDINE, PROPAMIDINE, DAPI, AND STILBAMIDINE IN BODY FLUIDS', Journal of Liquid Chromatography & Related Technologies, 24: 1, 29 — 45
To link to this Article: DOI: 10.1081/JLC-100000324
URL: http://dx.doi.org/10.1081/JLC-100000324

J. LIQ. CHROM. & REL. TECHNOL., 24(1), 29–45 (2001)

OPERATING PARAMETER EFFECTS IN CAPILLARY ZONE ELECTROPHORESIS (CZE): ANALYSIS OF PENTAMIDINE, HYDROXYSTILBAMIDINE, PROPAMIDINE, DAPI, AND STILBAMIDINE IN BODY FLUIDS

B. Rabanal, E. de Paz, N. Walser, and A. Negro*

Area de Química Analítica, Facultad de Biología,
Universidad de León, E-24071 León, Spain

ABSTRACT

A study was undertaken of the effects of varying those parameters that most affect analyses by capillary zone electrophoresis (CZE), electrolyte buffer, concentration of electrolyte, pH, voltage, temperature, and diameter of capillary, in respect to the following aromatic diamidines of therapeutic value: pentamidine, hydroxystilbamidine, propamidine, 6-amidino-2-(4-amidinophenyl) indole dilactate (DAPI), and stilbamidine. The data obtained permit the design of specific analytic methodology for each of these chemicals under differing conditions. As a demonstration, an analytical method is proposed in which all these chemicals are analyzed, simultaneously, in serum and urine under the following CZE conditions: citrate buffer 25 mM, pH = 3.75, $T = 30^{\circ}$C, and $V = 14$ kV. The detection limits for the method for serum and

*Corresponding author.

urine are (μg/mL): pentamidine, 0.3; hydroxystilbamidine, 0.4; propamidine, 0.2; DAPI, 0.3; and stilbamidine, 0.3. Oasis HLB cartridges were used for prior treatment of both serum and urine samples.

INTRODUCTION

In capillary electrophoresis, the various substances present in a sample are separated through the effects of a strong electric field, which produces differences in migration times. Over the time span of the analysis this leads to an effective separating out of each of them.

Several electrophoretic techniques exist that are used in capillaries for analytical purposes. Capillary zone electrophoresis (CZE) is the simplest working mode of those employed in capillary electrophoresis (CE). In it, separation is achieved in a buffer solution by applying a given voltage across the ends of the capillary tube. An electric field of constant strength is set up and the various analytes separate out thanks to their differing charge/mass ratios, yielding an electropherogram with different migration times (t_m) for each analyte.

Electro-osmotic flow (EOF) is an important effect occurring in CZE. It arises from the fact that at the interface between a solid and liquid an electric double layer is formed (1,2). The capillaries used in CZE are made of fused silica, and their inner surface is negatively charged at high pH values as a consequence of the protolysis of the silanol (silica hydroxide) groups. These negative charges on the capillary wall are counterbalanced by positive ions in the diffuse layer of the liquid. When an electric field is applied, there is movement of all the electrolytes contained in the capillary toward the cathode, dragging with it all the analytes present, even those with no charge, at different electrophoretic velocities (3,4).

The mobility of a bulk solution can be described as follows:

$$\mu_{\mathrm{EOF}} = \frac{\varepsilon E \zeta}{4\pi\eta}$$

where ε = dielectric constant of the solution, E = the electric potential applied, ζ = zeta potential, and η = viscosity of the solution.

Electrophoretic mobility (μ_e) may be expressed as follows:

$$\mu_e = \frac{q}{6\pi\eta r}$$

where q = charge of the ion and r = Stokes radius.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

The apparent mobility (μ_{app}) of the analyte is the algebraic sum of electrophoretic mobility (μ_e) and solution mobility (μ_{EOF}). This apparent mobility can be calculated using the equation:

$$\mu_{app} = \frac{L_d}{t_m E} = \frac{L_d L_t}{t_m V}$$

where L_d = length of the capillary to the detector, L_t = total length of the capillary, t_m = migration time, E = electric field, and V = applied voltage.

The process taking place in CZE is complex, so to permit good separation of the analytes in a sample, a large number of variables are involved, such as electrolyte buffer used, electrolyte concentration, electrolyte pH, voltage applied, temperature, diameter of the capillary, and so forth.

The aim of this work is to study operational parameter effects in CZE to gather data about the behavior of various chemicals in CZE and, hence, to achieve an optimal CZE analytic method for serum and urine to trace the following aromatic diamidines (Fig. 1), which are of considerable interest because of their chemical-therapeutic activity (5,6):

Pentamidine: 4,4′-[1,5-pentanediylbis(oxy)]bis-benzenecarboximidamide.
Hydroxystilbamidine: 4-[2-[4-(aminoiminomethyl)phenyl]ethenyl]-3-hydroxybenzenecarboximidamide.
Propamidine: 4,4′-[1,3-propanediylbis(oxy)bis-benzenecarboximidamide.
6-Amidino-2-(4-amidinophenyl)indole dilactate (DAPI).
Stilbamidine: 4,4′-(1,2-ethenediyl)bis-benzenecarboximidamide.

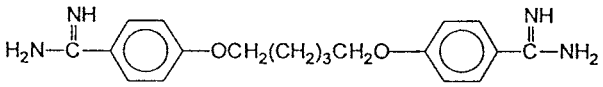
EXPERIMENTAL

Equipment

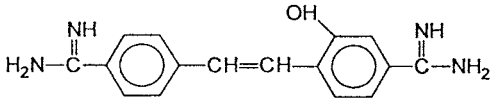
All the experimental work was carried out with a P/ACE System 2000 high-performance capillary electrophoresis setup (Beckman Coulter, Palo Alto, CA, USA). An untreated fused-silica capillary tube (Beckman Coulter) was used with a 75 μm internal diameter, L_t = 570 mm, L_d = 500 mm, enclosed in a liquid-cooled cassette. Detection was performed with a ultraviolet-visible (UV-VIS) detector, λ = 200 nm. Equipment was checked and data were processed with the software system Gold Nouveau (Beckman Coulter).

In preparing samples, the following were used: manifold (Waters, Milford, MA, USA), Concentrator Speed Vac Plus (Savant Instruments, Inc., Farmingdale, NY, USA).

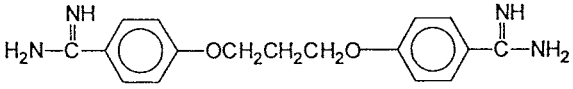
MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



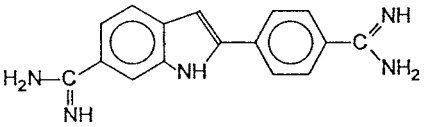
PENTAMIDINE



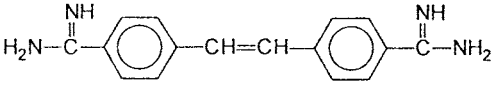
HYDROXYSTILBAMIDINE



PROPAMIDINE



DAPI



STILBAMIDINE

Figure 1. Structures of aromatic diamidines. Pentamidine: 4,4′-[1,5-pentanediylbis (oxy)]bis-benzenecarboximidamide. Hydroxystilbamidine: 4-[2-[4-(aminoiminomethyl) phenyl]ethenyl]-3-hydroxybenzenecarboximidamide. Propamidine: 4,4′-[1,3-propanediyl-bis(oxy)]bis-benzenecarboximidamide. DAPI: 6-amidino-2-(4-amidinophenyl)indole dilactate. Stilbamidine: 4,4′-(1,2-ethenediyl) bis-benzenecarboximidamide.

Reagents

Pentamidine and DAPI were obtained from Sigma-Aldrich Química S.A. (Madrid, Spain). Hydroxystilbamidine, propamidine, and stilbamidine were

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

generously given by Rhône Poulenc Rorer (Dagenham, UK). The solid-phase extraction system used was Oasis HLB cartridges (Waters).

Preparation of Standard Solutions

The standard solutions for each chemical were prepared by dissolving in pure water the requisite quantity of pentamidine, hydroxystilbamidine, propamidine, DAPI, or stilbamidine. These standard solutions were kept refrigerated in total darkness at 4°C.

Preparation of Serum and Urine Samples

Serum was prepared from human blood by centrifuging. Varying amounts of each chemical under study were dissolved in the serum to obtain the concentrations desired. Each of the chemicals was dissolved in human urine in the amount needed to obtain the concentrations required.

Conditioning of the Oasis HLB cartridge is carried out by passing it through 1 mL of methanol and then 1 mL of water. It is then loaded with 0.5 mL of urine or serum and washed with 1 mL of methanol/water 5%, with elution of the chemical with 1 mL of methanol, which will be evaporated in the Speed Vac and reconstituted in the same way as previously before starting analysis by CZE. In this case, it is necessary to add 10 μL of phosphoric acid per mL to the serum samples before initiating the process of extraction to prevent loss of the chemicals under test through bonding with proteins.

Electrophoretic Procedures

To achieve good repeatability, it was necessary for the process to be carried out with the capillary in identical conditions on all occasions. To achieve this, an unchanging work routine had to be adopted, including a cycle of washing and renewal of the previously used capillary for every electrophoretic process. The steps in the process used were regeneration of the capillary with NaOH 0.1 *M* (2 min), filling the capillary with work buffer (2 min), introduction of the sample into the capillary under pressure at 0.5 p.s.i. for 5 s, introduction of the buffer into the capillary under pressure at 0.5 p.s.i. for 1 s, placing the ends of the capillary into two vials with fresh buffer for each 10 processes to avoid "buffer depletion" (7,8), and initiation of the electrophoretic process. When the electrophoretic process was over, the capillary was washed with water for 2 min.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

RESULTS AND DISCUSSION

Choice of Electrolyte Buffer

In CZE the choice of electrolyte buffer must be carefully considered, as it is essential for achieving good analytic methodology. The electrolyte is generally composed of an aqueous solution of a buffering substance. In CZE it is vital to maintain pH constant throughout the whole procedure, because if this is not done the degree of ionization of the analytes, the EOF, and all the other analytic parameters can vary with it. The electrolyte has to be a good conductor of electricity if electrophoresis is to occur. The substances used as electrolytes are buffers that must have a sound capacity to regulate pH in the range $pK_a \pm 1$, low absorbency in UV-VIS, and low conductivity to minimize the intensity of current and the occurrence of Joule heating (9,10) and so permit high voltages giving low t_m and high efficiency.

The mobility of the buffer used should be similar to that of the analytes for the electropherogram peaks to be symmetrical (11). In this work, the most frequently used buffers with acidic pK_a (phosphate, acetate, citrate, and formate) were tested, and the best results regarding efficiency and resolution (Fig. 2) were obtained with citrate buffer. This buffer has the disadvantage that it has relatively high absorbency in the range of 190–230 nm obliging work to be done at low concentrations.

Concentration of Electrolyte Buffer

The concentration of the electrolyte is directly related to the ionic strength, and on it depend, in great measure, the parameters defining a good separation in capillary electrophoresis: migration time and mobility, separation selectivity, separation efficiency, and peak resolution. The concentration of the electrolyte buffer is linked to the majority of the parameters involved in the electrophoretic process according to the following equation (12):

$$I = \frac{\pi r^2 \varepsilon c \zeta}{\eta}$$

where I = intensity current, r = radius of capillary, ε = dielectric constant, ζ = zeta potential, η = viscosity of electrolyte, and c = electrolyte concentration.

To study the influence of electrolyte buffer concentration on the analytes under consideration, solutions of citrate electrolyte buffer were prepared at the following concentrations: 10, 20, 25, 30, 40, and 50 mM. With pH = 3.75, $V = 14$ kV, and $T = 30^\circ$C, electropherograms were produced for all the analytes concerned at each of these concentrations. Values for μ_{app} were obtained as represented in

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

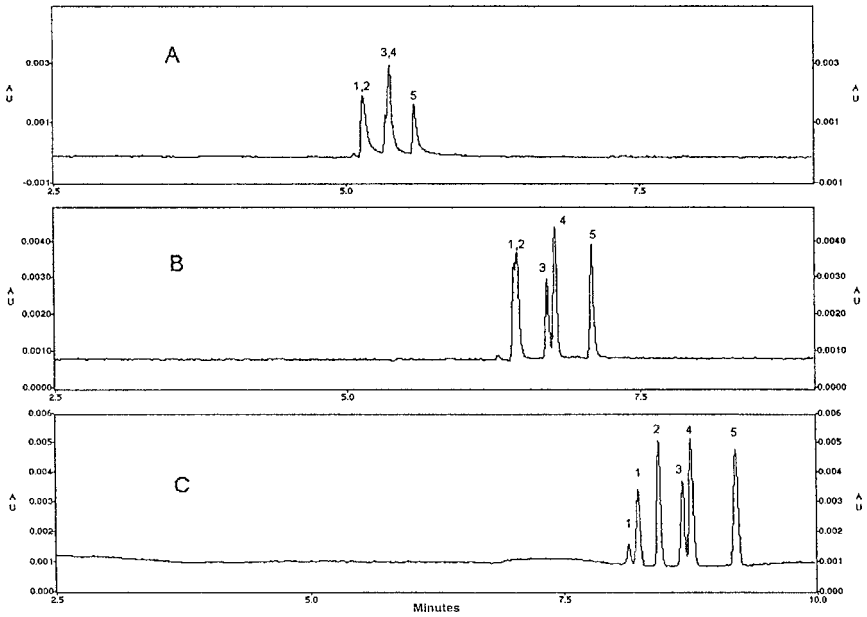


Figure 2. Electropherograms obtained using various electrolyte buffers: A) phosphate; B) acetate; C) citrate. Conditions: pH = 3.75, electrolyte buffer concentration 25 m*M*, voltage 14 kV. 1) stilbamidine; 2) DAPI; 3) hydroxystilbamidine; 4) propamidine; 5) pentamidine.

Figure 3, where it is very clear how these drop for all the analytes in question as electrolyte buffer concentration rises, above all, in the step up from 10 to 20 m*M*.

Electrolyte Buffer pH

Electrolyte buffer pH is probably the parameter with the greatest influence over whether a good CZE analysis is achieved, as pH is responsible for the electric charge on the analytes and hence for their electrophoretic mobility. If the sample contains a complex mixture or several analytes, it is advisable to start pH optimization trials at a value close to 7.

The pH value also influences the charge on the walls of the capillary tube which are composed of fused silica and have ionizable silanol groups in contact with the electrolyte buffer. The pH of fused silica is about 1.5; the degree of ionization of the wall of the capillary and EOF (13) is controlled by the pH of the electrolyte buffer.

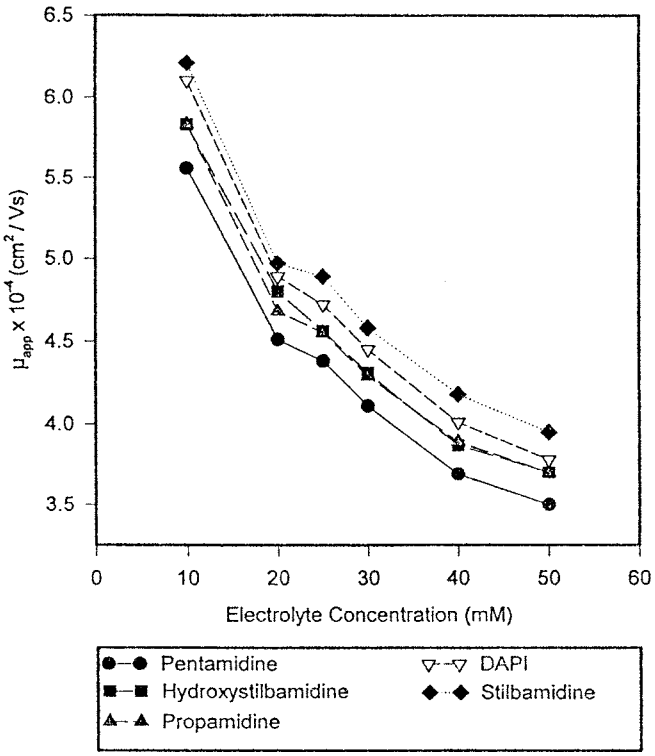


Figure 3. Variation in μ_{app} with variations in the concentration of the electrolyte buffer. CZE conditions: 25 m*M* citrate buffer; pH = 3.75; 14 kV; $T = 30^{\circ}$C; electrolyte concentrations 10, 20, 30, 40, 50 m*M*.

Variations in pH are also related to the production of Joule heat (14), as an increase in pH brings about a greater intensity of current, owing to a decrease in the resistance of the electrolyte, since there are more ions at higher pH values. Once the optimum pH has been selected for each case, it is necessary to maintain a constant temperature throughout the CZE procedure, since a small variation in temperature can give rise to variations in pH sufficient (15) to bring about significant modification in the CZE analysis conditions.

The strategy for pH optimization consisted of determining μ_{app} for each chemical in 25 m*M* citrate buffer with $V = 14$ kV, $T = 30^{\circ}$C, and successive pH levels of 3.25, 3.50, 3.75, 4.00, 4.25, 4.50, and 4.75. The values obtained are shown in Figure 4. It will be observed that all the chemicals evinced a similar behavior, a slight decrease in μ_{app} as pH went from 3.25 to 4.00, and then a sharp increase in μ_{app} with pH levels going from 4.00 to 4.75. This behavior is due, fundamentally,

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

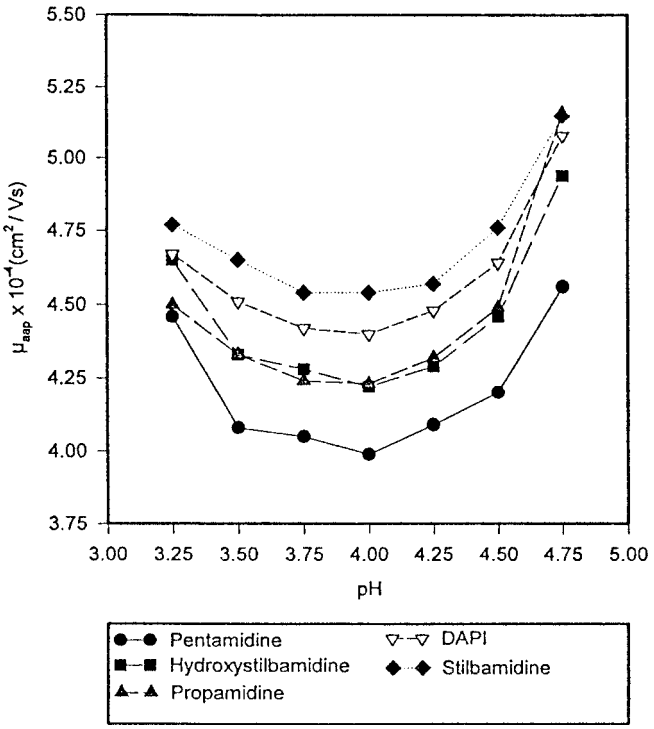


Figure 4. Variation in μ_{app} with variations in the pH of the electrolyte buffer. CZE conditions: 25 m*M* citrate buffer; pH = 3.25, 3.50, 3.75, 4.00, 4.25, 4.50, and 4.75, $V = 14$ kV; $T = 30°C$.

to the different degree of ionization of the diamidine groups present in the analytes as the pH of the electrolyte buffer varies.

It can further be noted from Figure 4 that, at certain pH levels, the values for μ_{app} of several analytes are very similar, so that it would not be possible to work at these pH levels with samples containing all the substances concerned, because the electropherogram peaks appear very close to one another. In this work it was observed that with a pH less than 4.1 the stilbamidine peak splits; this becoming more striking as pH decreases, so that it would be advisable to carry out analyses at pH 4.1 for this compound.

Applied Voltage

Control and optimization of the voltage used can result in an analysis being quicker and more effective with better-resolved peaks (13), because peak resolution

is proportional to the square root of the voltage applied. This means that to double the resolution the voltage applied has to be quadrupled. This is a severe requirement that limits the value of increases in voltage for CZE. For technical reasons most CE equipment operates with an upper voltage limit of 30 kV. A further problem is Joule heating (16) with increasing voltage, which can downgrade the analysis. Hence, although a voltage increase can, within certain limits, improve peak resolution, care must be taken not to exceed a reasonable voltage limit.

To determine the most appropriate voltage for analysis of these chemicals, each was tested to find its μ_{app} using 25 m*M* citrate buffer with pH = 3.75 and $T =$ 30°C and varying voltages of 8, 11, 14, 17, 20, 23, and 26 kV. The results obtained are shown in Figure 5, where it may be observed how all the analytes behave similarly, remaining practically constant up to 14 kV, rising sharply between 14 and 20 kV, and staying virtually constant from 20 kV upward.

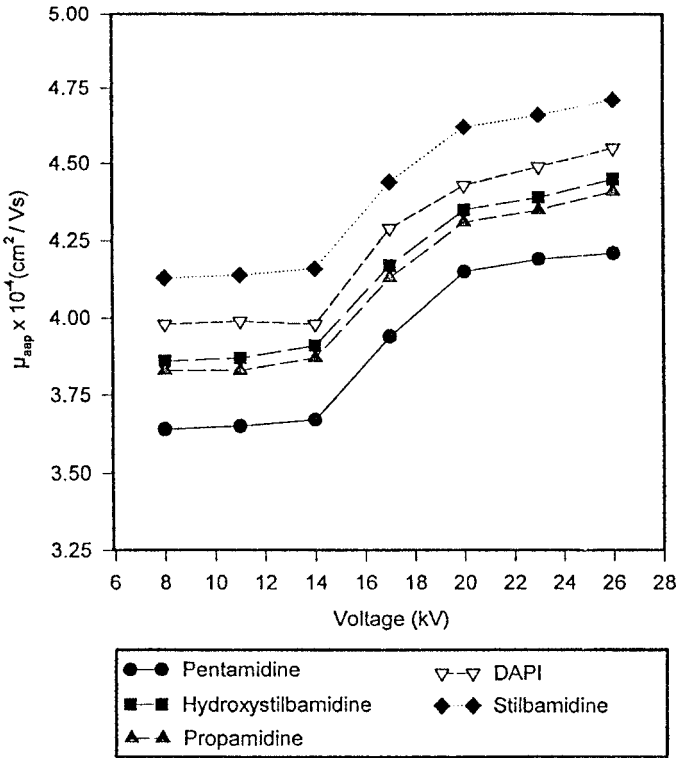


Figure 5. Variation in μ_{app} with applied voltage. CZE conditions: 25 m*M* citrate buffer; pH = 3.75; $T = 30$°C; $V = 8$, 11, 14, 17, 20, 23, and 26 kV.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Temperature

Precise temperature control during the CZE process is of great importance for achieving good separation selectivity, and above all, good reproducibility of results (17). In technical terms a number of different methods are used to maintain a constant temperature in the system: thermostat-controlled ovens, high-velocity gas flows, and so forth. The equipment used in this study maintained a constant temperature in the capillary by means of circulating cooling liquid in direct contact with the capillary.

Some simple transformations of the Gibbs-Helmholtz equation (18),

$$\Delta G = \Delta H - \Delta T S,$$

where ΔG is Gibbs free energy, ΔH is the enthalpic (heat content) term, ΔS is the entropic term of the free energy, and T is the absolute temperature, give the equation,

$$\ln \alpha = -\Delta\left(\frac{\Delta H}{RT}\right) + \Delta\left(\frac{\Delta S}{R}\right),$$

where α is the selectivity of separation.

As this equation shows, separation selectivity will decrease with an increase in temperature. Thus, if selectivity is insufficient for a given separation problem, the analysis can be performed at a lower temperature to increase selectivity. This is certainly true for thermodynamically controlled processes. If in a given separation system the selectivity is high enough to allow adjustments, a higher temperature may be used to improve the peak form, to shorten the analysis time, or to achieve some special temperature-dependent effects, such as conformational and configurational charges or dynamic CZE.

To determine the influence of temperature on the process of analysis by CZE, all the chemicals were analyzed using 25 mM citrate buffer with pH = 3.75 and $V = 14$ kV at various temperatures of 28, 31, 34, 37, and 40°C. On the basis of the electropherograms, the μ_{app} was calculated for each chemical at each temperature and the data obtained are shown in Figure 6.

It may be observed that they all have very similar patterns. This was to be expected, as their chemical structures are quite alike. It can also be noticed that, as temperature goes up, there is a noteworthy increase in the values for μ_{app}. In addition, it can be seen how hydroxystilbamidine and propamidine can only be separated in samples containing both from 38 to 40°C, since up to these temperatures the values for their μ_{app} are very close.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

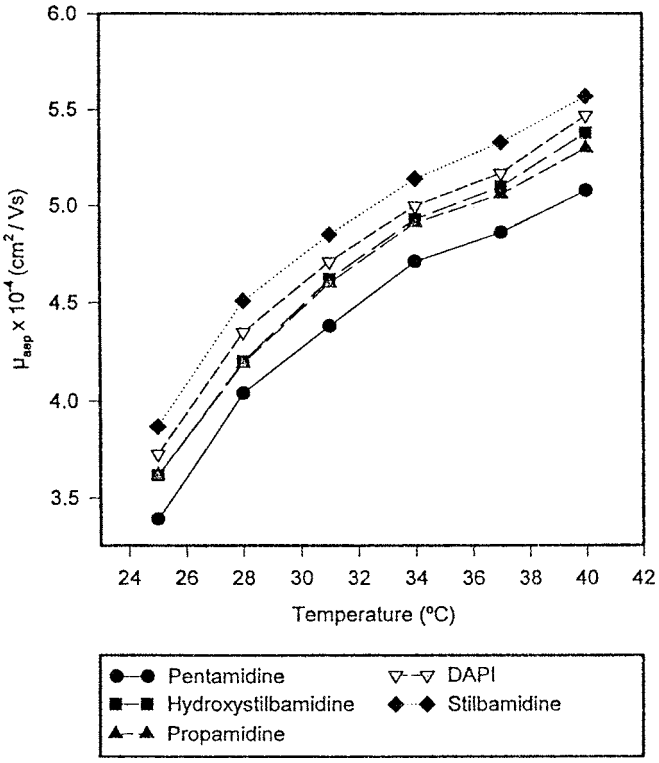


Figure 6. Variation in μ_{app} with variations in temperature. CZE conditions: 25 m*M* citrate buffer; pH = 3.75; $V = 14$ kV; $T = 25$, 28, 31, 34, 37, and 40°C.

Diameter of Capillary

As capillary diameter is increased, larger quantities of sample can be loaded, and the sensitivity of the analytic method grows (19,20). Moreover, it is easier to align the capillary in the detector window and an improvement in signal to noise ratio occurs. This effect can be observed in Figure 7, which shows electropherograms using capillaries of 50 μm (A) and 75 μm (B) diameter. Sample injection took place under pressure in both instances. In the 50-μm capillary the injection time was 15 s, but 5 s in the 75-μm capillary. It can be seen how the signal to noise ratio is much higher in the 75-μm capillary. To attain such a ratio in the 50-μm capillary, it would be necessary to use very long injection times, with consequent loss of efficiency. As for resolution, a slight improvement occurs in the 50-μm capillary.

A further effect that is detected as capillary diameter goes up is an increase in the heat generated. The intensity of the current is directly proportional

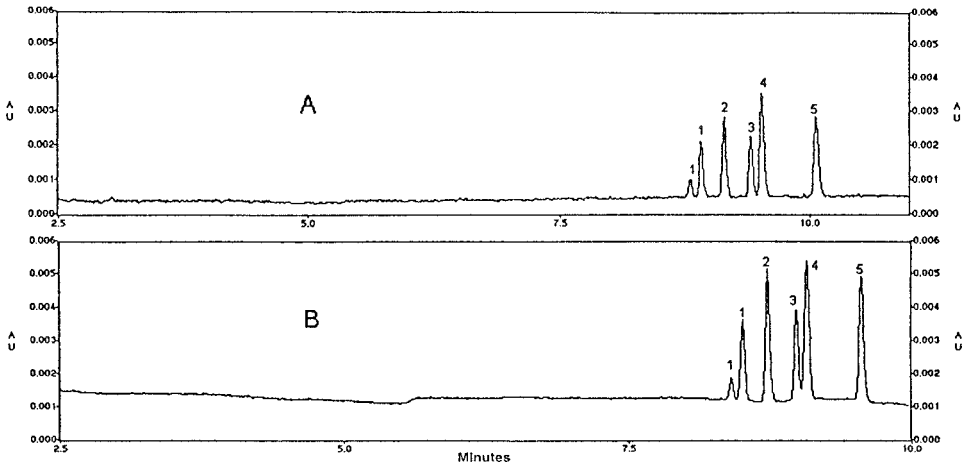


Figure 7. Electropherograms obtained on capillaries of different diameters. A) capillary diameter 50 μm; B) capillary diameter 75 μm. CZE conditions: 25 m*M* citrate buffer; pH = 3.75; $T = 30^{\circ}$C; $V = 14$ kV. 1) stilbamidine; 2) DAPI; 3) hydroxystilbamidine; 4) propamidine; 5) pentamidine.

to the square of the capillary diameter (18),

$$\Delta T = \frac{0.24 W r^2}{4 K_{\mathrm{T}}},$$

where W = power, K_{T} = thermal conductivity of the buffer, and r = capillary radius.

This renders temperature control more difficult with capillaries of larger diameter. In this experiment, 25 m*M* citrate buffer was used with pH = 3.75, $V = 14$ kV, and $T = 30^{\circ}$C. A current intensity of 23.6 μA was attained with a 75-μm capillary and 10.8 μA with a 50-μm capillary. In both instances, the heat generated is perfectly absorbed by the cooling system of the equipment, with capillary temperature being held constant.

Most Appropriate Conditions for Analysis

The data obtained from the studies detailed above permit a deep acquaintance with the behavior of this family of chemicals in CZE. As a result, it is possible to predict the most appropriate conditions for analyzing these substances in serum and urine. The conditions in question are electrolyte, 25 m*M* citrate buffer, pH = 3.75, $T = 30^{\circ}$C, and $V = 14$ kV.

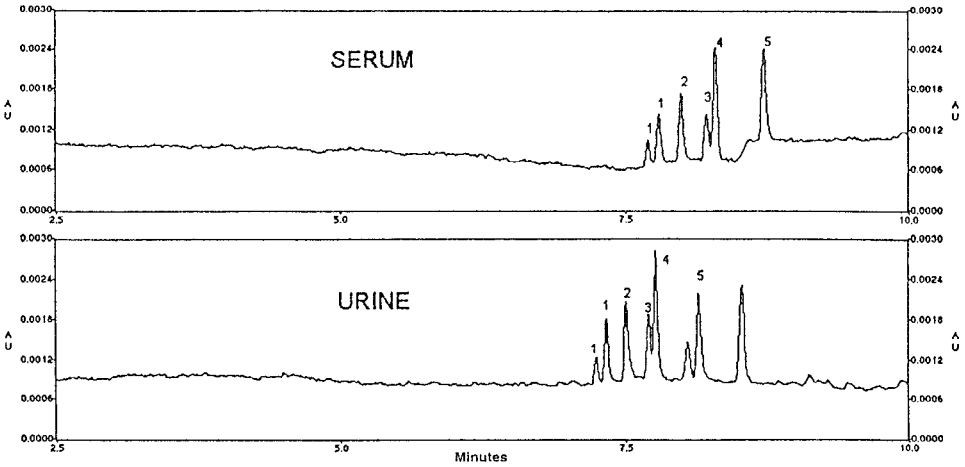


Figure 8. Electropherograms of serum and urine. CZE conditions: 25 m*M* citrate buffer; pH = 3.75; $V = 14$ kV; $T = 30^{\circ}$C; concentration of analytes 2.75 μg/mL. 1) stilbamidine; 2) DAPI; 3) hydroxystilbamidine; 4) propamidine; 5) pentamidine.

Under these conditions the following electropherograms were obtained for serum and urine in a sample, in which all the chemicals were supposed to be present together (Fig. 8).

Evaluation of the Method

Samples of differing concentrations diluted in water were analyzed for each of the substances. The data obtained were processed using linear regression, and the figures given in Table 1 were the outcome.

Six samples of serum and urine supplemented with each of the chemicals were prepared to achieve final concentrations in serum and urine of 1.75 and 2.75 μg/mL. The process of extraction was carried out using Oasis HLB

Table 1. Data for Evaluation of Method: Linearity

	Equation	Correlation Coefficient
Pentamidine	$y = 0.0004665x + 0.2441$	0.999
Hydroxystilbamidine	$y = 0.0004785x + 0.862$	0.998
Propamidine	$y = 0.0004664x + 0.003442$	0.999
DAPI	$y = 0.000466x + 0.2818$	0.997
Stilbamidine	$y = 0.0005723x + 0.1041$	1

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Table 2. Data for Evaluation of the Method: Recovery Test

	Serum			Urine		
	Conc. (μg/mL)	Recovery (%)	CV (%)[a]	Conc. (μg/mL)	Recovery (%)	CV (%)
Pentamidine	1.75	98.7	2.3	1.75	81.2	2.2
	2.75	95.7	2.7	2.75	82.1	2.3
Hydroxystilbamidine	1.75	97.1	1.1	1.75	93.1	2.0
	2.75	90.5	2.4	2.75	80.7	2.6
Propamidine	1.75	91.2	1.8	1.75	83.2	0.9
	2.75	85.1	2.2	2.75	92.4	2.1
DAPI	1.75	80.3	3.2	1.75	78.4	2.4
	2.75	81.2	4.1	2.75	77.7	3.4
Stilbamidine	1.75	85.3	1.9	1.75	80.4	3.2
	2.75	84.2	2.7	2.75	87.2	3.1

[a]CV, coefficient of variation.

extraction cartridges and each sample was analyzed, yielding the results shown in Table 2.

The detection limits μg/mL were determined to be: hydroxystilbamidine, 0.3; propamidine, 0.2; DAPI, 0.3; and stilbamidine, 0.3. Interassay precision was determined by replicate analyses as 1.75 ($n = 6$) and 2.75 μg/mL ($n = 6$). Table 3 shows that the percentage coefficient of variation for both of these concentration ranges was between 1 and 0.997.

Table 3. Data for Evaluation of the Method: Precision

	Level (μg/mL)	Calculated Average	SD	%CV
Pentamidine	1.75	1.84	0.03	3.5
	2.75	2.75	0.09	9.4
Hydroxystilbamidine	1.75	1.77	0.03	3.1
	2.75	2.75	0.09	9.1
Propamidine	1.75	1.83	0.02	2.9
	2.75	2.77	0.09	9.1
DAPI	1.75	1.90	0.07	7.6
	2.75	2.79	0.08	8.4
Stilbamidine	1.75	1.78	0.04	4.7
	2.75	2.74	0.08	8.0

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

CONCLUSIONS

Several of the most important parameters influencing the analysis of these substances in CZE were studied. Using the data obtained, it is possible to become familiar with the behavior of each chemical when analyzed using CZE. This will permit the design of analytic methods using CZE for all of the substances best matching the needs for each. On the basis of the data obtained in this study, an analytic method can be proposed as an example for samples of serum and urine in which all the chemicals are present. The most suitable conditions for such an analysis by CZE are 25 m*M* citrate buffer, pH = 3.75, $T = 30^{\circ}$C, and $V = 14$ kV. Under these conditions, a rapid, precise, and reliable analytic method is achieved, which is an alternative to other methods for analysis of these substances using high-performance liquid chromatograghy.

ACKNOWLEDGMENTS

We thank Rhône Poulenc Ltd. for their generous gift of all the substances studied in this paper. The work was carried out with the equipment in the Instrumental Techniques Laboratory of the University of León.

REFERENCES

1. Hunter, R.J. *Zeta Potential in Colloid Science*; Academic Press: London, 1981.
2. Hjerten, S. Chromatogr. Rev. **1967**, *9*, 122.
3. Kuhn, R.; Hoffstetter-Kuhn, S. *Capillary Electrophoresis: Principles and Practice*; Springer: Heidelberg, 1993.
4. Jorgenson, J.W.; Lukacs, K.D. Anal. Chem. **1981**, *53*, 1298.
5. Pegg, A.E. Biochem. J. **1986**, *234*, 249.
6. Pegg, A.E. Cancer Res. **1988**, *48*, 759.
7. Strege, M.A.; Lagu, L. J. Liq. Chromatogr. **1993**, *16*, 51.
8. Landers, J.P.; Oda, O.P.; Madden, B.; Sismelich, T.P.; Spelsberg, T.C. J. High Resolut. Chromatogr. **1992**, *15*, 517.
9. Hjerten, S. Chromatogr. Rev. **1967**, *9*, 122.
10. Knox, J.H. Chromatographia **1988**, *26*, 329.
11. Kohlrausch, F.K. Ann. Phys. (Leipzig) **1987**, *62*, 209–239.
12. Cohen, A.S.; Paulus, B.; Karger, B.L. Chromatographia **1987**, *24*, 15–24.
13. Jorgenson, J.W.; Lukacs, K.D. Anal. Chem. **1981**, *53*, 1298–1302.

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

14. Rush, R.S.; Cohen, A.S.; Karger, B.L. Anal. Chem. **1991**, *63*, 1346–1350.
15. Terabe, S.; Yashima, Y.; Tanaka, N.; Araki, A. Anal. Chem. **1988**, *60*, 1673–1677.
16. Knox, J.H.; Grant, I.H. Chromatographia **1987**, *24*, 135–143.
17. Nelson, R.J.; Paulus, A.; Cohen, A.S.; Guttman, A.; Karger, B.L. J. Chromatogr. B **1989**, 11–127.
18. Chankvetadze, B. *Capillary Electrophoresis in Chiral Analysis*; Wiley & Sons: New York, 1997.
19. Wang, T.; Hartwick, R.A.; Champlin, P.B. J. Chromatogr. **1989**, *462*, 147–157.
20. Tsuda, T.; Sweedler, J.V.; Zare, R.N. Anal. Chem. **1990**, *62*, 2149–2152.

Received June 18, 2000 Author's Revisions August 20, 2000
Accepted July 13, 2000 Manuscript 5330

Request Permission or Order Reprints Instantly!