ELSEVIER

Journal of Chromatography A, 828 (1998) 317–336

JOURNAL OF CHROMATOGRAPHY A

Review

Analysis of nucleotides by capillary electrophoresis

Susan E. Geldart, Phyllis R. Brown*

Department of Chemistry, University of Rhode Island, Kingston, RI 02881, USA

Abstract

Capillary electrophoresis is a useful tool for the analysis of nucleotides. Methods have been optimized for both CZE and MECC modes. A variety of CZE buffers, such as borate, carbonate and phosphate were used successfully. The pH of the buffer changes the charge on the nucleotides. Therefore, the selectivity of the analytes can be controlled by the acidity of the buffer solution. CE separations of nucleotides have been performed at all pH levels, in both CZE and MECC modes. SDS was the most commonly used modifier in MECC separations, but other additives have been added to optimize selectivity. In addition, nucleotides have been quantified in different matrices, including tissue and cell extracts and several DNA and RNA sources. This paper summarizes the methods used for the optimization of nucleotides by CE and includes the most recent techniques to improve selectivity, reproducibility and sensitivity. A summary of CE methods is used in analyses of nucleotides in biological matrices is included.

Keywords: Reviews; Nucleotides

Contents

*Corresponding author. Tel.: +1 401-874-2282, Fax: +1 401-874-5072.

0021-9673/98/$ – see front matter
PII: S0021-9673(98)00633-5

1. Introduction

There is a critical need for nucleotide analyses in many fields. Nucleotides are both precursors and break-down products of DNA and RNA and they are intrinsically involved with cell metabolism [1–16]. Nucleotide mutations have been linked to cancer and genetic diseases [1–5,17]. Since some nucleoside analogs are effective drugs in treating AIDS, cancer, and other diseases their triphosphate metabolites must frequently be monitored [1,6–10]. Thus, analyses of nucleotides in body tissues and cells, are widely used in biochemical, medical and pharmacology studies. In addition, nucleotides can produce ''off-flavors'' in foods and their concentrations must be determined for quality control in the food industry [13].

Nucleotides have been quantified by high-performance liquid chromatography (HPLC) for approximately 30 years. While HPLC analyses are effective, they have limitations. The columns are expensive and large amounts of the mobile phase are needed for the analyses. The sample volume required is relatively large, often between 10–100 μl. Column equilibration in the ion-exchange and ion pairing modes can be time consuming, with a total analysis time of nearly an hour [1,2,6–9]. When radioactive tracers are used to attain the desired sensitivity, solvent disposal and personnel training costs are high [6,7,10–12].

Nucleotides are easily separated by capillary electrophoresis (CE) because of their negative charge. Capillary electrophoretic methods are broadly classified into two main categories; capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC or MEKC). Since two other modes of separation, capillary gel electrophoresis (CGE) [39] and isotachophoresis (ITP) [47] are not widely used in the separation of nucleotides, the focus of this review will be on separations by CZE and MECC.

CE analyses are generally faster than comparable HPLC analyses, the solvents used are inexpensive buffer salts and smaller quantities of both buffer and sample are required. CE is a flexible technique with several separation and detection modes. Currently researchers have used both MECC [8,14,15,17–27] and CZE [1,2,4–6,9,12–14,16,18,28–44] modes to separate nucleotides. As with HPLC, a variety of detection methods, including UV [1,2,5,6,8,9,12–16,19–37], fluorescence [4,18,38–40], mass spectrometry (MS) [5,17,42,43], and electrochemical [33,43], methods have been used to quantify and/or identify nucleotides and their analogs.

2. Nucleotide structure and charge calculations

2.1. Nucleotide structure

Nucleotides are easily analyzed by CE methods because they are negatively charged in a pH range from 2–12. Both the structures and the net ionic charges on the molecule play a part in the selectivity of the compounds. The structure of a nucleotide can be divided into three parts; a purine or pyrimidine base, a ribose sugar and from one to three phosphate groups (Fig. 1).

The bases most commonly analyzed are the purine and pyrimidine compounds associated with DNA and RNA. The bases, adenine, cytosine and guanine are the same in both DNA and RNA. In DNA, the fourth base is thymine, and in RNA its complement is uracil. In addition to these bases, two other nucleotides, inosine monophosphate and xanthosine monophosphate are frequently included in nucleotide analyses (Fig. 2).

A nucleoside consists of a ribose sugar added to the 9 carbon on a purine or the 3 carbon on a pyrimidine. The nucleoside is in the ribose form if there are hydroxyl groups in the 2′ and 3′ positions. The deoxyribose nucleoside only has a hydroxyl in the 3′ position of the ribose (Fig. 1).

In a nucleotide there are one to three phosphate groups in the 2′, 3′, or 5′ positions on the ribose of

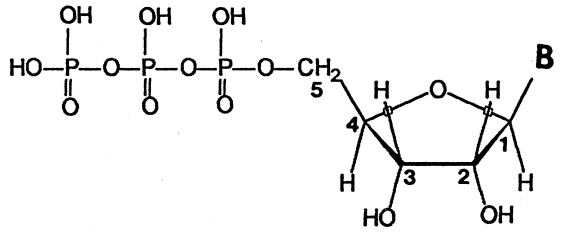

Fig. 1. Nucleotide structure: triphosphate shown. B = Purine or pyrimidine base.

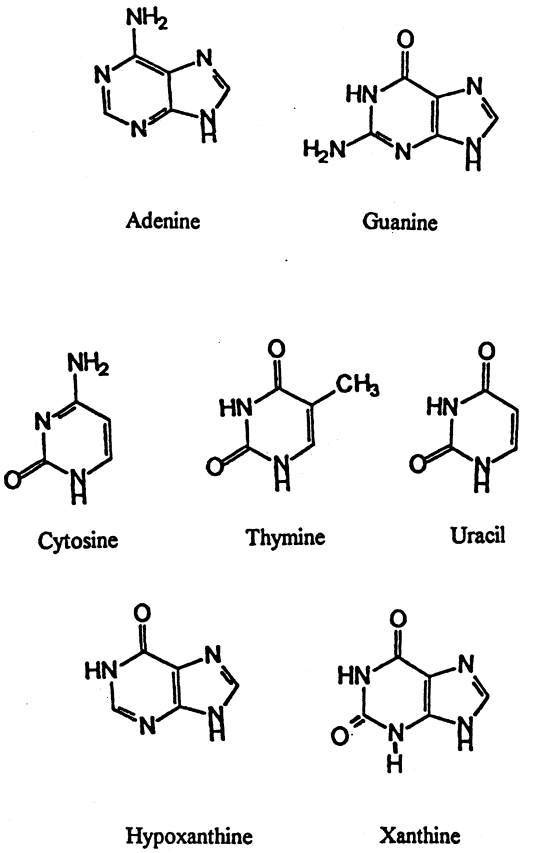

Fig. 2. Structures of the purine and the pyrimidine bases of nucleotides analyzed by CE.

the nucleoside. Above pH 6, each phosphate adds an additional negative charge to the nucleotide; thus a nucleoside triphosphate such as adenosine triphosphate, ATP, has a higher charge than its mono- or diphosphorylated forms.

2.2. Nucleotide charge

The overall charge on a nucleotide can be derived from the Henderson–Hasselbalch equation for acids:

$$\mathrm{pH} = \mathrm{p}K_a - \log\,(1/a - 1) \qquad (1)$$

The charge on a nucleotide is complicated because there are several pK_a values associated with each nucleotide [46]. The charge is based on the number of phosphate groups present and the partial ionic charges from the purine or pyrimidine base and the sugar. The total charge on the nucleotide is calculated by adding the partial ionic charges determined from the Henderson–Hasselbalch equation for each pK_a value [46]. The pK_a values associated with the ionization of the phosphate, base and sugar are listed in Table 1.

The ionization of the first phosphate occurs at such a low pH, below 1, that the nucleotide is charged throughout the pH range of 20-12. Each additional phosphate group has a pK_a in the range of 6–7; thus at neutral or high pH, the di- and triphosphorylated forms of the nucleotides will have a higher overall charge than a monophosphate.

The charge on the purine or pyrimidine base that is determined by the pH of the buffer is primarily responsible for the selectivity of the separation between nucleotides that have the same number of phosphate groups [46]. Since there is a wide range in ionization constants for the base compounds, base pK_a can be used in the selection of a buffer.

Table 1
Ionization constants of nucleotides

Nucleoside base	Phosphate form	Base pK_a	Secondary phosphate
Adenosine	5′		6.4
	2′		6.17
	3′		5.92
Deoxyadenosine	5′		6.65
Cytidine	5′		6.62
	3′		6.04
Guanosine	5′	9.24	6.66
	2′	9.6	N/A
	3′	9.4	5.92
Deoxyguanosine	5′	9.7	6.4
	3′	9.7	6.4
Uridine	5′	9.5	6.4
	3′	9.43	5.88
Thymidine	5′	10	6.5
Inosine	5′	8.9	6.04
Xanthosine	5′	5.7, 11.1	N/A

Data from Ref. [59].

The dissociation of the hydrogen from a sugar moiety occurs at a pH greater than 12. Because a pH range above 12 is higher than would normally be used in a CE separation, very little of the overall charge is due to the ribose portion of the molecule. While there are slight differences in the pK_a values associated with the deoxyribose versus the ribose form of the nucleotide, the bulk of the charge on the nucleotide in the pH range of 2 to 11 is from the base and phosphate groups. Therefore the overall negative charge on a nucleotide provides the basis for separation by CE.

3. Optimization of nucleotide analyses by CE

To develop and optimize CE analyses of nucleotides, it is necessary to have an understanding of the underlying principles of CE separations. Therefore, a brief presentation of the theory and methods involved in nucleotide separations will be presented. Several reviews and books are available which provide more detailed discussions of CE theory [14,48–53].

Method optimization involves maximizing the experimental properties of selectivity, reproducibility, and sensitivity. Selectivity, which primarily involves attaining the maximum resolution between analytes is important when large numbers of analytes are to be separated in a sample. Reproducibility is needed especially when routine analyses are performed and sensitivity is required for the analysis of samples with low analyte concentrations. The theory and experimental applications relating to each of these optimization parameters will be presented.

3.1. Selectivity

The goal of a selective analysis is baseline, or near baseline separation of all the analytes of interest. Buffer composition causes changes in both selectivity and resolution. Buffer pH plays a significant role in CE because charge differences between analytes drive electrophoretic based separations. However, buffer composition, especially in the MECC mode is also important. The interaction of solutes with the buffer may result in a changes in migration times and elution order. Thus, the selectivity and resolution of a separation can be controlled by the careful selection of buffer pH, buffer salts and modifiers. CE separations of nucleotides have been performed in both CZE and MECC modes under a variety of conditions.

3.2. CZE

CZE separations are based on the relative migration of the negatively charged analytes to the positively charged anode and the attraction of the ions in the buffer for the negatively charged cathode. The overall mobility of the electroosmotic flow (EOF) must be greater than that of the analytes, or the analyte cannot be detected. Theoretically the velocity of the analyte is

$$v = \mu E \tag{2}$$

where v is the ion velocity, μ is the electrophoretic mobility and E is the applied electric field [48]. The fastest ions will be those with the highest mobility and those exposed to the highest voltage. The term μ, is related to ionic charge and the ionic radius based on the following equation:

$$\mu = q/6\lambda\eta r \tag{3}$$

where q is the ion charge, n is the solution viscosity and r, the ionic radius. Under the same buffer conditions of concentration, composition and pH, μ is proportional to q/r [48].

The effective ionic mobility may be calculated experimentally from the following equation;

$$\mu_e = L_t L_d / V(1/t_a - 1/t_{eof}) \tag{4}$$

where L_t is the total capillary length, L_d, the effective capillary length (length of capillary to the detector), V, the applied voltage, t_a, the migration time of the analyte and t_{eof}, is the migration time of the EOF. The resulting calculation yields a negative value for mobility, because of the negative charge on the nucleotide [48]. As the nucleotide moves against the EOF, the least mobile species are eluted first, and smaller, more negatively charged species are detected last when bare capillary columns are used.

The selectivity of nucleotide separations by CZE is based on buffer pH and composition. Buffer pH is important, as it determines the ionic charge on each nucleotide; thus the migration order expected at pH 3

would be different from that achieved with a buffer at pH 11. However, nucleotide separations by CZE have been successfully performed under acidic, neutral and basic conditions. For the separation of nucleotides, acidic buffers have been used by many researchers [2,21,28,30–33,37,41]. The buffers found to be effective in the separation of nucleotides in the pH range of 2–6 included salicylic acid/sodium salicylate [37], acetic acid [31], ammonium acetate [41], sodium phosphate [21], sodium formate [28] and Tris–HCl [2,32,33]. Modifiers such as EDTA, acetonitrile or methanol had to be added to obtain adequate resolution.

The use of a neutral buffer for nucleotide separations by CE has been reported. In 1988, Tsuda et al. [34] used sodium phosphate buffers in the range of 6–8 to separate 5′-ribonucleotides in guinea pig organs. Dawson et al. [22] also used a sodium phosphate buffer with a pH of 6.6 in a separation of impurities from the phosphonate analogue of ATP, FPL 67085XX. The addition of methanol and EDTA were needed to achieve adequate resolution of the nucleotide analog from its monophosphate, nucleoside and base.

Alkaline solutions are widely used buffers for nucleotide separations [1,2,4–6,12,16,18,19,29,30, 35,36,40,44,46,54]. Alkaline buffers provide a stable EOF; thus coated capillaries are not needed to eliminate or stabilize the EOF. Since the capillary is already preconditioned to an alkaline environment, capillary equilibration time after a NaOH rinse is minimal [1,6,46]. In addition, the EOF is fast, and higher buffer concentrations can be used to eliminate band broadening that occurs if the ionic strength of the sample matrix is higher than that of the buffer [5,6]. Because the second and third phosphate ionizations occur in the pH range of approximately 6–7, differences in migration time for monophosphorylated versus triphosphorylated forms of a nucleotide with the same base structure were higher under alkaline conditions than with an acidic buffer. In addition, alkaline buffers provide better separation of a monophosphorylated nucleotide from its di- and triphosphorylated forms due to the additional ionization that occurs above pH 6 (Table 1).

Sodium borate was the most commonly used alkaline buffer for nucleotide separations by CE [4,9,12,16,35,36,45]. Pentoney et al. [44] used a borate buffer, with a pH of 8.1 to separate radiolabeled AMP from ATP and GTP. Ng et al. [9] also used sodium borate but at a pH of 9.4, to separate 5′-ribonucleotides in human blood cells. The nucleotides were identified by spiking the sample with standard nucleotides and observing comigration. A 20 m*M* sodium borate buffer, pH 9.24, was used by Liu et al. [35] to analyze pig feed for IMP and GMP. While these researchers used sodium borate buffers successfully in their separations, other investigators found that a modifier was needed with the borate buffer to achieve adequate resolution. Li et al. [4] separated four fluorescein labeled deoxynucleotides, dAMP, dCMP, dGMP and dTMP with a 10 m*M* Tris–borate buffer at pH 8.7. The addition of 10% acetonitrile improved the peak shape. Although Uhrova et al. [36] used a phosphate/borate buffer to separate twelve 5′-ribonucleotides, Norwood et al. [16] determined that borate buffers alone provided inadequate resolution for the separation of the four ribonucleoside monophosphates and used sodium carbonate to separate DNA adducts.

While not as widely used as sodium borate, sodium and ammonium carbonate buffers are becoming more popular. Carbonate buffers provide a stable baseline and adequate resolution for many nucleotide analyses. Geldart and Brown [45] separated the twelve 5′- ribonucleotides with a 30 m*M* sodium carbonate/bicarbonate buffer at pH 9.5 with baseline resolution of all twelve peaks (Fig. 3). Finally, DeForce et al. [5] took advantage of the volatility of ammonium carbonate and interfaced a CE with MS for the separation and detection of DNA adducts.

Other buffers used for high pH separations of nucleotides include glycine [19], ammonium acetate [41], and 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) [13,43]. Tadey and Purdy [19] separated 12 2′,3′, and 5′-ribonucleoside monophosphates in 15 min with baseline resolution with a glycine buffer at pH 9 (Fig. 4). The addition of borate and β-cyclodextrin complexing agents were necessary to obtain good resolution. Wolf and Vouros [40] used a volatile ammonium acetate buffer in the analysis of DNA adducts by CE–MS and Luong et al. [43] and Nguyen et al. [13] used CAPS buffers to analyze for hypoxanthine, inosine and IMP in fish tissue.

Although the pH of the buffer is an important

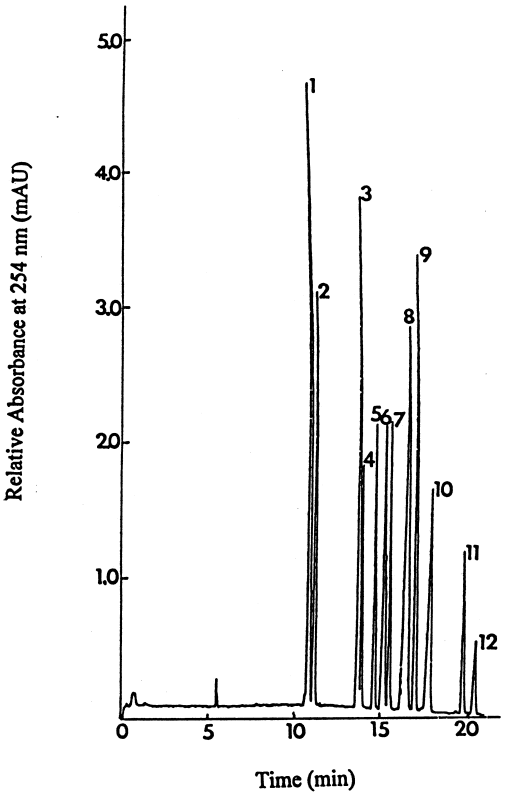

Fig. 3. CE separation of twelve 5′-ribonucleotides. The separation conditions were 30 m*M* sodium carbonate/bicarbonate buffer, pH 9.5, hydrostatic injection for 10 s, voltage 18 kV. The migration order of the nucleotides was; 1=AMP, 2=CMP, 3=ADP, 4=GMP, 5=CDP, 6=ATP, 7=UMP, 8=CTP, 9=GDP, 10=GTP, 11=UDP, and 12=UTP. Reprinted with permission from Ref. [45].

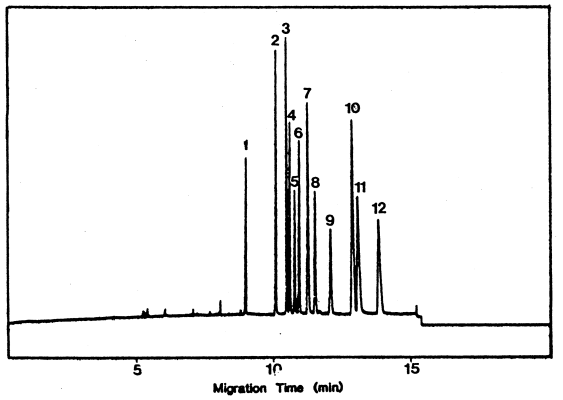

Fig. 4. CE separation of 12 nucleotides based on complexation with borate and β-CD, pH 9. Peaks: 1=3′-AMP; 2=2′-AMP; 3=3′GMP; 4=3′-CMP; 5=2′-CMP; 6=2′-GMP; 7=3′-UMP; 8=2′-UMP; 9=5′-AMP; 10=5′-GMP; 11=5′-CMP; 12=5′-UMP. Reprinted with permission from Ref. [19].

factor in the separation of nucleotides, the relationship between the experimental mobility and the theoretical mobility is not fully understood. The ionic charge, q, can be directly calculated from the pK_a of the analytes and the pH of the buffer using the Henderson–Hasselbalch equation [45]. The ionic radius is dependent on factors such as molecular mass and interactions of the solute with the buffer.

Differences in the migration order of nucleotides based on charge-to-molecular mass ratios have been reported [19,36,38,46]. In 1988, Kuhr and Yeung [38] determined that there was good agreement between elution order and the theoretical mobility calculated from the Henderson–Hasselbalch equation with a salicylate buffer at pH 3.5. They concluded that there were minimal interactions between the analytes and either the capillary wall or the buffer components under these conditions. In 1997, Geldart and Brown [45] determined that a linear relationship existed between experimental mobility and charge to mass ratio under alkaline conditions. The correlation between experimental mobility and q/M calculated using the Henderson–Hasselbalch equation for 12 5′-ribonucleotides was 0.974 when a 30 m*M* sodium carbonate/bicarbonate buffer at a pH 9.5 was used. They found similar correlations existed for other nucleotide analyses throughout the pH range of 9–11 and a buffer concentration of 30–50 m*M*. However, the migration order found by Uhrova et al. [36] when a phosphate/borate buffer in the same pH range was very different from that of Geldart and Brown (Fig. 5). The analysis with the carbonate buffer resulted in a migration order of monophosphates followed by diphosphates and finally the triphosphates. However, when a borate/phosphate buffer was used the migration order became monophosphates followed by triphosphates and finally diphosphates. Uhrova explained the unusual order as the result of incomplete ionization of the triphosphates as would be calculated from the Offord parameter expressed as the

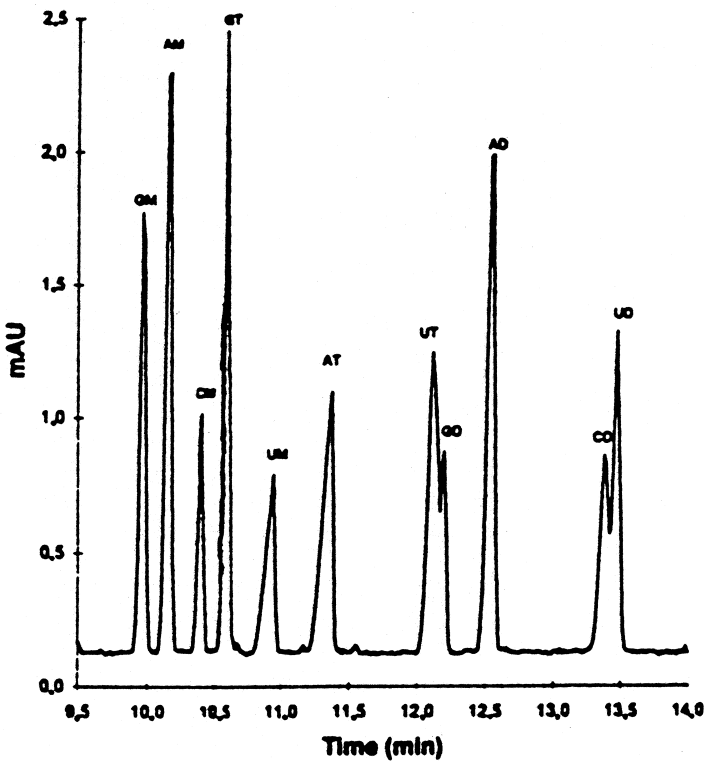

Fig. 5. Electropherogram of an 11-membered mixture of nucleoside-5′-phosphates. Conditions: individual sample concentration 10 μg/ml. Buffer: 0.02 *M* phosphate–borate buffer, pH 10, 70 cm capillary, voltage: 20 kV, 1 s hydrodynamic injection, detection at 254 nm. Peak identification: 1=GMP, 2=AMP, 3=CMP, 4=GTP, 5=UMP, 6=ATP, 7=UTP, 8=GDP, 9=ADP, 10=CDP, 11=UDP. Reprinted with permission from Ref. [36].

ratio of the molecular mass (M_r) of the solute and the effective charge (z) of the particle in the form $M_r^{2/3}/z$.

3.3. MECC

Buffer composition becomes more complex when MECC is used. Originally developed to separate neutral compounds by CE, MECC has also been found to be useful in the separation of charged compounds [48–53]. The addition of a surfactant, usually sodium dodecyl sulfate (SDS), to the buffer creates micelles within the buffer which act as a chromatographic stationary phase. Thus, in addition to the charge to mass based electrophoretic separation, chromatographic partitioning is also present. The capacity factor for the electrophoretic separation is determined from the following equation [48].

$$k' = (t_r - t_0)/t_0(1 - t_r/t_m) \quad (5)$$

where k' is the capacity factor, t_0 the migration time of an unretained solute, t_r, the migration time of the analyte, and t_m, the migration time of the micelle. The formula is similar to that of a chromatographic separation, except for the introduction of the migration time of the micelle, which is included to account for the movement of the ''stationary phase'' through the capillary.

MECC has been used in the separation of nucleotides by CE [8,14,15,17–27]. The most widely used buffer is a combination of phosphate and borate salts with 10–100 m*M* of SDS added as a surfactant

[15,17,20,22,23,25,27]. Plate counts for these analyses are often very high. Row and Raw [20] achieved 170 000 theoretical plates/m for a separation of four deoxyribonucleoside triphosphates and Wang et al. [24] separated four ribonucleoside monophosphates with a plate count of 65 000. An analysis of eight isomeric forms of 3′-ribonucleoside monophosphates and 2′-deoxy-3′-ribonucleoside monophosphates by Lecoq et al. [17] had a plate count of 237 000 theoretical plates/m. Thus, the addition of a chromatographic partitioning phase resulted in a separation of these isomeric compounds which were not fully resolved on a basis of electrophoresis alone.

Another advantage of MECC is that neutral and charged compounds can be simultaneously separated. MECC has been used to separate mixtures of neutral nucleosides and charged nucleotides. Row et al. [25] first used MECC in 1987 to separate four 2′-deoxy 5′-ribonucleotides from their 2′-deoxyribonucleosides. Lahey and St. Claire, III [23] separated five neutral deoxyadenosine and deoxyguanosine nucleosides and eight negatively charged deoxyadenosine and deoxyguanosine nucleotides from AZT, a nucleoside analog in approximately 40 min. With an optimized isocratic ion pairing reversed-phase liquid chromatography (RP-LC) method only half the compounds were resolved (Fig. 6a and b).

While SDS was the most commonly used surfactant in MECC separations, dodecyltrimethylammonium bromide (DTAB) was also used [8,35]. In addition to creating micelles, DTAB coats the capillary walls, causing the wall to be positively instead of negatively charged [8]. The positively coated wall results in a reversal of the EOF as the positively charged micelles now migrate toward the cathode, which is normally located at the buffer inlet. The voltage polarity was reversed to reestablish the EOF toward the detector. The addition of DTAB accomplished two goals; first, it minimizes the EOF, making the separation more reproducible, and second, it adds partitioning character to the buffer. Liu et al. [35] and Loregian et al. [8] both used buffers containing DTAB to separate nucleoside triphosphates.

In addition to the surfactant, buffer modifiers were used to increase resolution or change selectivity. Organic modifiers, such as isopropanol [20] and acetonitrile [15,17] were used to increase the speed of the analysis and obtain better peak shape. EDTA [8,12,22] was also used, especially with buffers containing borate, to enhance peak efficiency.

3.4. Reproducibility

The need for reliable, reproducible results is critical in most analyses. In general, CE analyses have been less reproducible than similar HPLC separations. However, with better understanding of CE methods and the use of coated capillaries, area and migration time reproducibility in the separations of nucleotides by CE was reported to be better than 1% [8,15,17,18,21].

The main causes of irreproducible results in CE are the methods of injection, interactions of the solute with the capillary wall, and build-up of wastes in the buffer vials. Both electrokinetic and hydrodynamic injection methods have been used in CE separations of nucleotides. While electrokinetic injection is preferred for higher sensitivity, the reproducibility is lower than when hydrodynamic injection is used [17,21,47]. Capillary conditioning with either HCl [20] or NaOH [1,2,4,5,8,17–19,36] was found to increase both resolution and reproducibility by providing the same surface conditions between runs. In addition, frequent replenishment of both buffer containers eliminated variations in ionic strength and volume level between the inlet and outlet buffer compartments resulting in more reproducible results [5].

Capillary wall modifications have been used to increase reproducibility. Silated capillaries, especially those coated according to the method by Hjertan [54], have been found to increase reproducibility [2,21,38]. Silylation also increased baseline stabilization, and decreased noise and peak broadening. Takigiku et al. [21] used silated capillaries to eliminate the EOF; thus the mobility of the analyte was no longer dependent on the EOF, resulting in a more reproducible separation.

Other methods of eliminating the EOF involved the addition of cetyltrimethylammonium bromide (CTAB) to the buffer. Huang et al. [28] separated ribonucleoside monophosphates from three different RNA sources with a sodium formate buffer containing CTAB. The separation was achieved in 5 min and the percentages of AMP, CMP, GMP and UMP

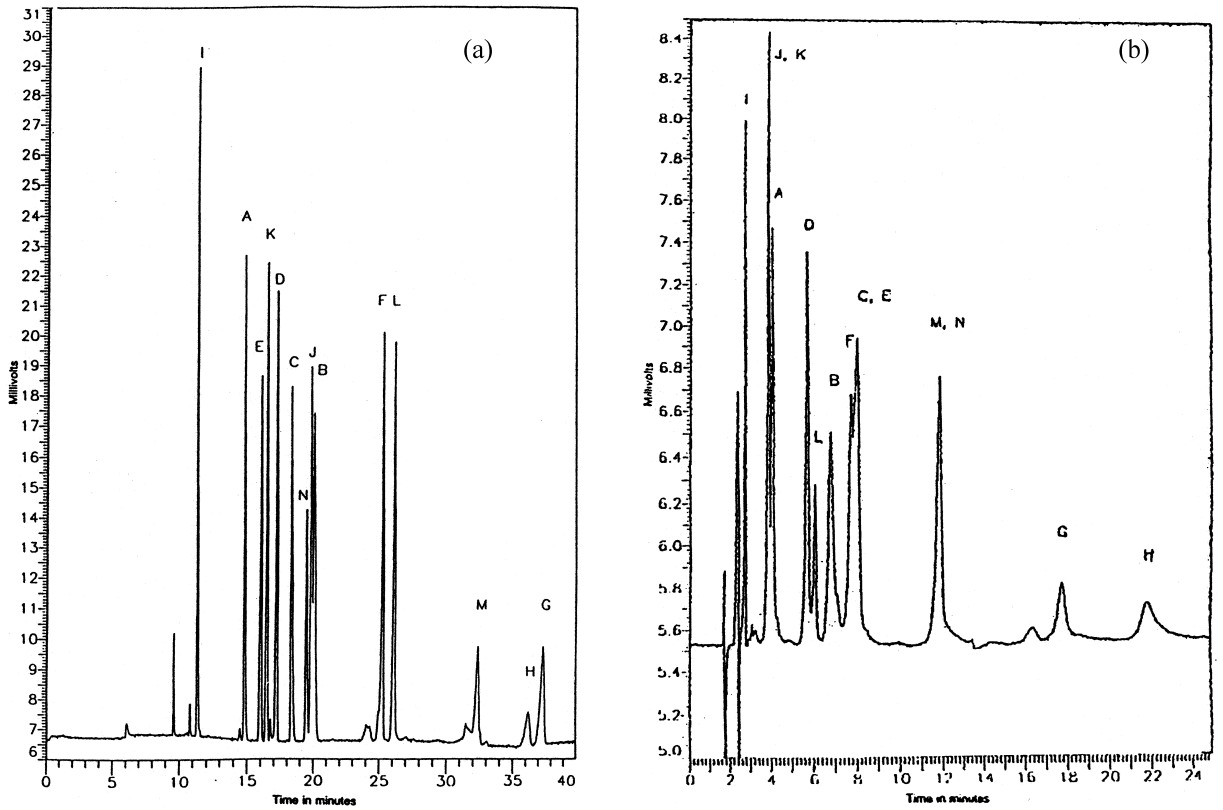

Fig. 6. (a) Separation by MECC. Conditions: individual sample concentration: 357 μ*M*, buffer: 20 m*M* sodium phosphate buffer, pH 7.1 with 200 m*M* SDS, 72 cm capillary, voltage: 20 kV, 1 s vacuum injection, detection at 252 nm. Peaks: (A) 5′-Deoxyadenosine, (B) 2′,3′-dideoxyadenosine, (C) N^6-methyl-2′-adenosine 5′-monophosphate, (D) 2′-deosyadenosine 5′-monophosphate, (E) N^6-methyladenosine 5′-monophosphate, (F) adenosine 5′-diphosphate, (6) 2′-deoxyadenosine 5′-triphosphate, (G) 2′-deoxyadenosine 5′-triphosphate, (H) 2′,3′-dideoxyadenosine 5′-triphosphate, (I) 2′-deoxyguanosine, (J) 2′,3′-dideoxyguanosine, (K) deoxyguanosine 5′-monophosphate, (L) 2′-deoxyguanosine 5′-diphosphate, (M) 2′,3′-dideoxyguanosine 5′-triphosphate, (N) 3′-azido-3′deoxythimidine (AZT). Reprinted with permission from Ref. [23]. (b) Separation by HPLC. Conditions: column: Adsorbosphere HS (C_{18}), 250×4.6 mm, column temperature: 35°C, mobile phase: 8% acetonitrile in 50 m*M* ammonium phosphate buffer with 2 m*M* tetrabutylammonium hydroxide, pH 6.5, injection volume: 50 μl, individual sample amount: 13–55 pmol. Buffer: 20 m*M* sodium phosphate buffer, pH 7.1 with 200 m*M* SDS, 72 cm capillary, voltage: 20 kV, 1 s vacuum injection, detection at 252 nm. Peaks as in (a). Reprinted with permission from Ref. [23].

found experimentally in each source matched the published values to within 1.4%.

UCON-coated capillaries were used to eliminate the EOF and to increase efficiency of ribonucleotide separations [32,33]. O'Neill et al. [33] separated 15 ribonucleotide standards in 45 min with a phosphate/Tris–HCl buffer at pH 5.2 (Fig. 7). The relative standard deviation (R.S.D.) of the migration time ranged from 0.21–0.52%. Shao et al. [32] achieved the same reproducibility with a UCON-coated column for a separation of 12 ribonucleotides in a lymphoma cell extract. The detection limits for these separations were approximately 10^{-6} *M*.

3.5. Sensitivity: detectors

The sensitivity for CE separations is determined primarily by the type of detection used. Several detectors, including UV [1,2,5,6,8,9,12–16,19–37], fluorescence [4,18,38–40], and MS [5,17,41,42] have been used in CE separations of nucleotides.

Two-thirds of the researchers reported the use of

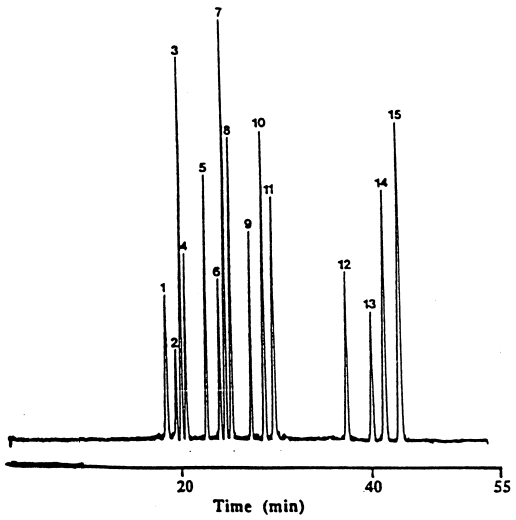

Fig. 7. CE separation of ribonucleotide standards on a UCON-coated column. Conditions: 90 cm×50 μm I.D. column, 54 cm separation distance; 30:50 m*M* phosphate–Tris–HCl buffer, pH 5.2; electromigration injection for 6 s at −23 kV; −20 kV applied voltage. Peaks: 1=UTP, 2=CTP, 3=ATP, 4=GTP, 5=UDP, 6=CDP, 7=ADP, 8=GDP, 9=XMP, 10=UDP-g, 11=ADP-r, 12=UMP, 13=CMP, 14=AMP, 15=GMP. Reprinted with permission from Ref. [33].

UV detection in the CE analyses of nucleotides. There are several advantages to UV detection. First, it is inexpensive and easy to use. The instrumentation is relatively simple and UV detection technology has already been used extensively for HPLC. The flow cell on most CE instruments with UV detection is the capillary itself [48]. The UV detector is situated perpendicularly to the capillary; thus the pathlength of the flow cell is the same length as the inner diameter of the capillary. UV detection is highly sensitive to mass limit of detection and detection limits of picomoles and occasionally femtomoles have been achieved [15,17,23,33,34]. The calibration curves generated with UV detection are linear over two- to three-orders of magnitude and the sample volume required was 1 to 10 nl.

Although the UV range of 250–280 nm has been used for nucleotide analyses by CE–UV, 254 nm was the most commonly used wavelength. Samples analyzed with UV detection include nucleotides [1,2,8,9,12,19,23,31–33,36], DNA adducts [5,16,17,22], and drug metabolites [6,23]. MECC was frequently used to separate mixtures of bases, nucleosides and nucleotides in the same sample [16,23,27]. No differences were seen in the detection limits for MECC and CZE separations. Detection limits for most nucleotide separations by CE were in the picomole range [15,17,23,33,34].

While the mass limit of detection is excellent for CE analyses of nucleotides, the concentration limit of detection was inadequate for many applications because of the small pathlength of the flow cell. According to Beer's Law

$$A = \epsilon bC \tag{6}$$

As the pathlength *b*, gets smaller, the concentration of the solution, *C*, must be higher to generate sufficient absorbance, *A*, to generate a detectable signal. In CE separations, the absorbance signal detected is limited by the pathlength, which is typically 25–75 μ [48]. As a result, detection limits achieved for nucleotide analyses by CE were in the micromolar range [1,2,6,12,16,17,21,24,26,32,34]. While this limit was sufficient for many analyses where nucleotides were abundant, the sensitivity was not high enough to analyze for drug metabolites or adducts that require detection at lower concentrations [5,6,16,41].

Thus, more sensitive detection methods were required for many nucleotide analyses. Fluorescence, especially laser-induced fluorescence (LIF) methods were found to be very successful in decreasing detection limits for nucleotide analyses. Kuhr and Yeung [38] used an indirect method to analyze 5′-monophosphate and diphosphate nucleosides. The high background fluorescence of the sodium salicylate buffer used resulted in detection limits of 70 attamoles for each of the 5′-nucleoside monophosphates detected. The advantages of an indirect fluorescence method were higher sensitivity than with UV detection and no sample derivatization.

While indirect fluorescence methods are more sensitive than similar UV analyses for nucleotides, sample derivatization with fluorescent tags resulted in more sensitive CE analyses for nucleotides. A variety of different compounds, including fluorescein [4], and choloroacetaldehyde [18] were used to create fluorescent nucleotide derivatives. Tseng et al. [18] used a LIF method to separate derivatized

adenine compounds from oocytes. The concentration limit of detection was in the nanomolar range, with a mass limit of detection of $5 \cdot 10^{-19}$ moles for the adenine nucleotides. The method was linear over five-orders of magnitude and the separation of the nucleotides was achieved in under 10 min (Fig. 8).

In addition to fluorescence and UV techniques, several other detection methods have been used in the analysis of nucleotides by CE. Pentoney et al. [44] took advantage of the high sensitivity of radioactivity detection methods and developed a radioisotope detector for a CE system. Detection limits of 10^{-9} *M* were achieved for ^{32}P labeled nucleotides. The detection limit was lowered 10-fold if a flow programming procedure was incorporated into the analysis.

Krattinger et al. [27] developed a hologram based thermooptical absorbance detector for CE analyses. The MECC separation of a mixture of nucleosides and nucleotides was accomplished in 20 min (Fig. 9). The detection limit for AMP was 50 n*M*, and no sample derivatization was required.

The coupling of CE with wall-jet amperometric detection was achieved by Lin et al. [42] for the separation of nucleoside monophosphates. The method was linear over two- to three-orders of magnitude and the detection limit for all analytes was below 9 fmoles. The method was successfully applied to the separation of nucleotides and nucleosides in plasma.

The interface of CE with mass spectrometry is the most active area of research in the CE field. CE–MS has been used for the analysis of nucleotides and DNA adducts but the technique is still in its infancy [5,17,40,41]. Fast atom bombardment (FAB) MS [17,40] was the most frequently used mode of MS for CE–MS systems, although DeForce et al. [5] successfully separated DNA adducts with an electrospray (ESI) method (Fig. 10). Both MECC and CZE methods have been used in CE–MS techniques, but the SDS used in MECC separations was found to be incompatible with both fast atom bombardment (FAB) and ESI detection methods [17,41]. In addition, buffer additives such as polyvinyl alcohol, or Triton X-100, used to eliminate the EOF reduced the sensitivity of the method [41] thus the buffer used in CE–MS separations must be carefully selected. CZE separations of DNA adducts with ammonium carbonate [5] and ammonium acetate [40] buffers have been performed, but the sensitivity of the analyses was limited. Preconcentration methods, including capillary isotachophoresis (cITP) [41] and sample stacking [5,40] were needed for the analysis of DNA adducts in biological matrices.

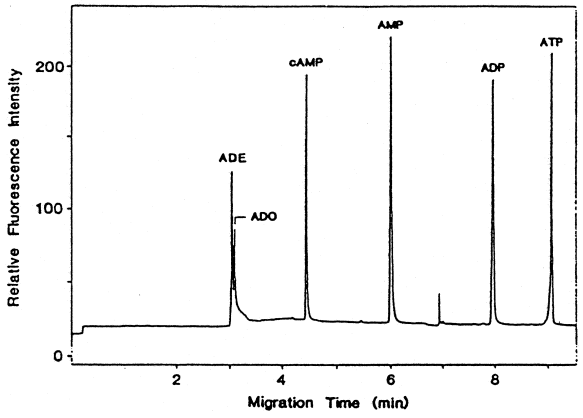

Fig. 8. Electropherogram of approximately 2 μ*M* each of adenine (ADE), adenosine (ADO), cAMP, AMP, ADP and ATP derivatized with chloroacetaldehyde and analyzed with confocal laser-induced fluorescence apparatus. The peak between AMP and ADP is an unknown impurity peak. Column: 60 cm×25 μm I.D., 20 m*M* sodium phosphate buffer, voltage: 20 kV. Reprinted with permission from Ref. [18].

3.6. Sensitivity: preconcentration

Due to the relatively high detection limits of many of the current CE detection techniques, including UV and MS, sample preconcentration has been used to increase sensitivity [5,6,16,40,41]. On-line preconcentration methods included cITP [41] and sample stacking [5,6,40]. Reversed-phase cITP was used by Zhao et al. [41] in the CE–MS separation of four ribonucleotide monophosphates. These preliminary results were encouraging and future plans involve the application of this method for the characterization of adducts that result from radiation induced DNA damage.

Analyte ''stacking'' is a phenomenon specific to CE separations [45,55–59]. Stacking of an analyte is caused by a difference in ionic strength between the sample and the buffer [57]. As the analytes move through the capillary, they are separated into discreet

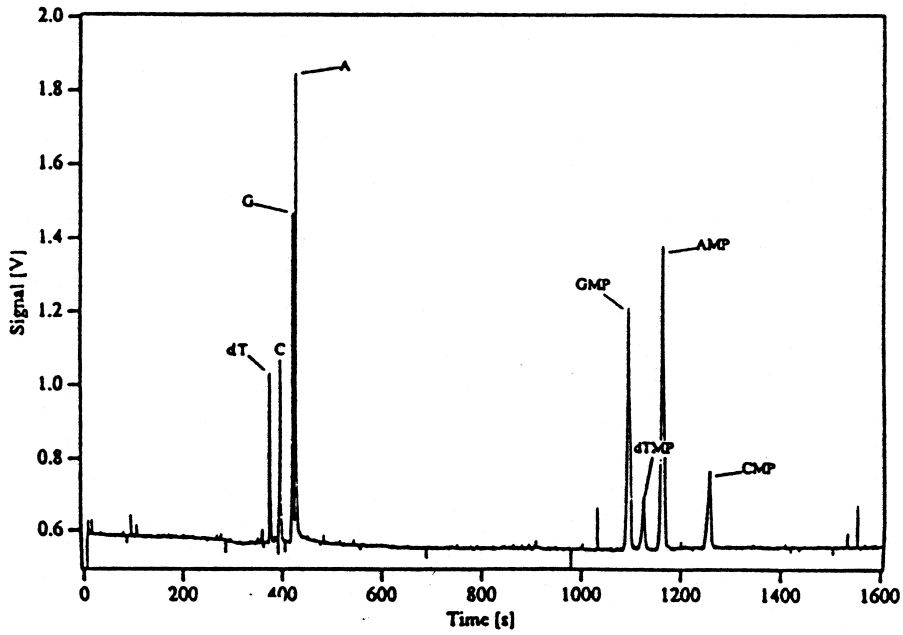

Fig. 9. MEKC electropherogram of a mixture of nucleosides and their monophosphate nucleotides detected by thermooptical absorbance. Conditions: column: 75 cm×20 μm I.D.; buffer: 20 m*M* sodium phosphate, 20 m*M* sodium tetraborate, 50 m*M* laural sulfate, pH 7, detection: 257 nm, voltage: 30 kV, injection: 30 s pressure of 25 mbar. Reprinted with permission from Ref. [27]. ©1996 American Chemical Society.

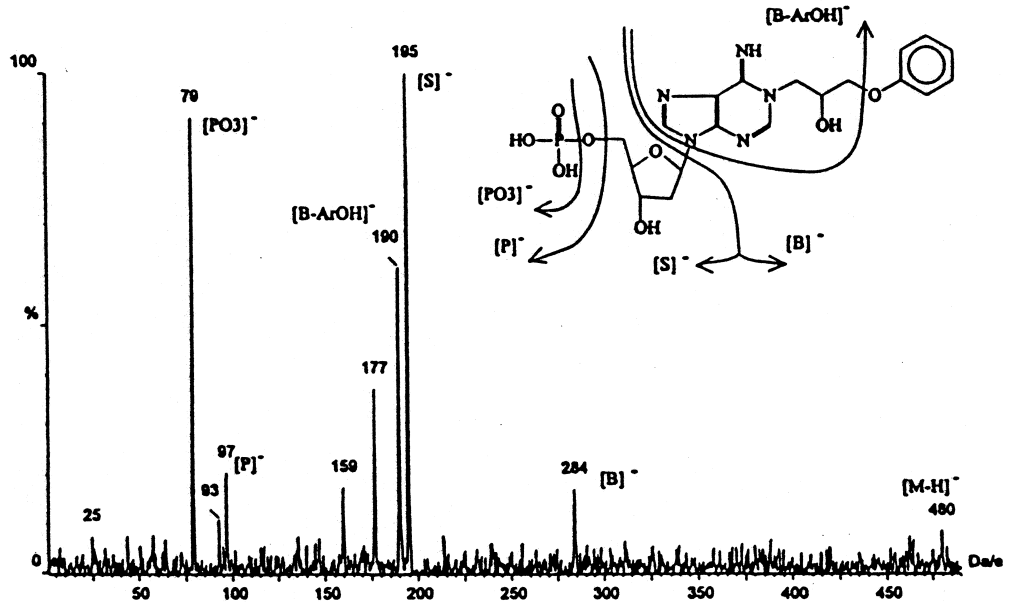

Fig. 10. CZE–ES-MS–MS spectrum of the daughters monoalkylated dAMP (mother ion mass $[M-H]^- = 480$), present in the hydrosylate of the reaction of DNA with PGE. Here the sample stacking technique was used following an injection of 100 mbar for 30 s. The collision energy was set at 25 eV. Reprinted with permission from Ref. [5]. ©1996 American Chemical Society.

zones that have the same ionic strength as the buffer. If the ionic strength of the sample is lower than that of the buffer, the analytes will be drawn into a very tight narrow zone in the capillary (Fig. 11). Conversely, if the ionic strength of the analytes is greater than that of the buffer, then band broadening occurs as the sample components spread out to match the lower ionic strength of the surrounding buffer. Thus, the resolution and sensitivity of the CE method can be modified by changes in the ionic strength of the buffer and that of the sample matrix [5,6,40].

Three types of sample stacking have been performed in CE separations of nucleotides. Ionic strength mediated stacking has been observed when the sample matrix has a lower ionic strength than the surrounding buffer. This phenomenon has not only occurred naturally in nucleotide separations [2,21,33,35], but has also been used deliberately as a method of sample preconcentration [5,6,40]. The use of ionic strength mediated stacking increases sensitivity by a factor of 10 when compared to a separation without stacking [54,56].

The use of electrokinetic injection for sample introduction has been associated with an increase in sensitivity of a CE separation [45]. Further sensitivity enhancement can be achieved by hydrodynamically injecting a small plug of water onto the capillary before an electrokinetic injection is made. Electrokinetic stacking occurs as the analytes move quickly

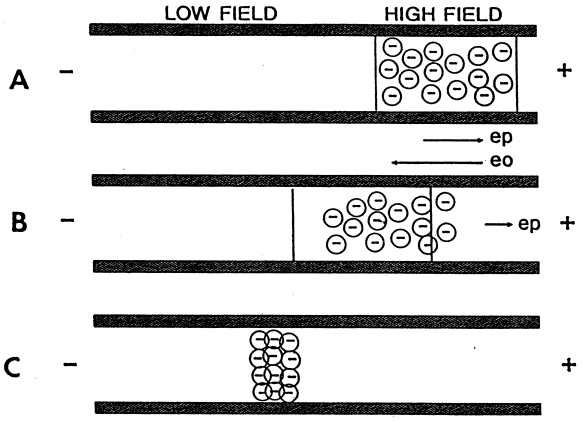

Fig. 11. Illustration of ionic-strength mediated stacking of anions in a high-pH buffer. eo: Electrophoretic velocity of the buffer, ep: electrophoretic velocity of the anion. (A) Sample is loaded on to the capillary. (B) Voltage is applied and anions move through the sample zone toward the cathode. (C) Samples approach the buffer/sample interface, slow down and are dragged toward the anode by the EOF. Reprinted with permission from Ref. [47]. ©1993 Academic Press.

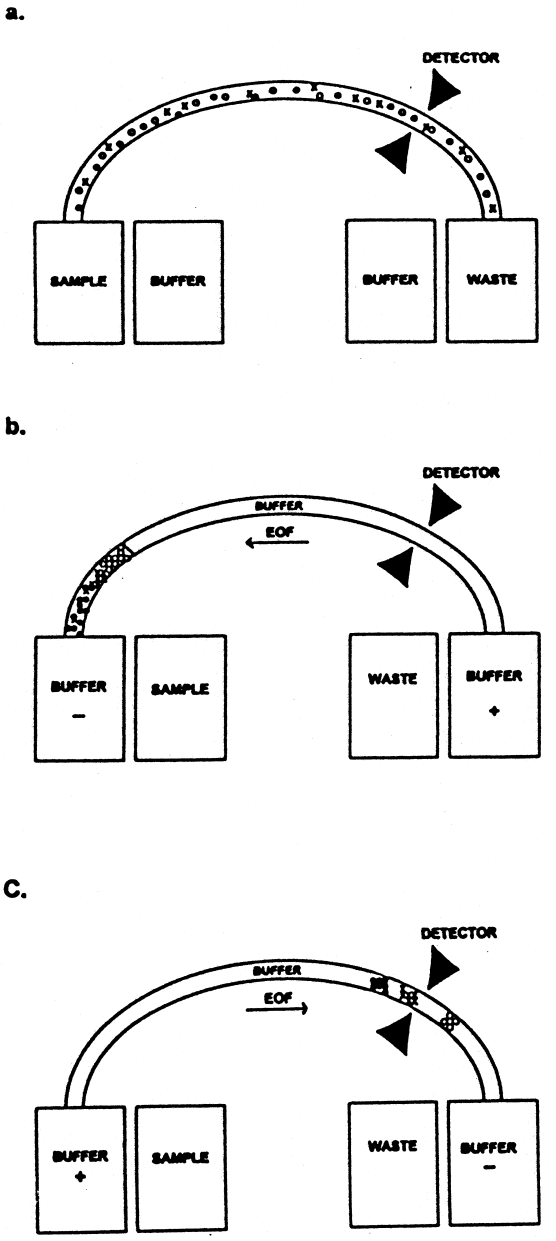

Fig. 12. Steps involved in whole capillary stacking. (A) Sample is loaded onto the capillary. (B) Sample stacking and matrix removal. Negative voltage applied. (C) Analytical separation. Positive voltage applied. Reprinted with permission from Ref. [6]. ©1998 John Wiley and Sons, Inc.

through the low ionic strength of the water and stack in discreet narrow zones at the buffer/sample interface. Electrokinetic stacking is associated with >10-fold increases in sensitivity for many analyses.

The most effective stacking method used for improving the sensitivity of CE separations of nucleotides is whole capillary stacking, or whole capillary injection. Whole capillary stacking methods increase the sensitivity of CE techniques by injecting large quantities of sample onto the capillary, or completely filling the capillary [1,5,6,16,40,56–59]. Band broadening associated with large sample volumes is minimized by the removal of the sample matrix before analysis. The procedure is fast; the injection and sample removal steps take between 5–8 min, and the separation is similar to an analysis performed without preconcentration [6,54]. The steps involved with whole capillary stacking are shown in Fig. 12.

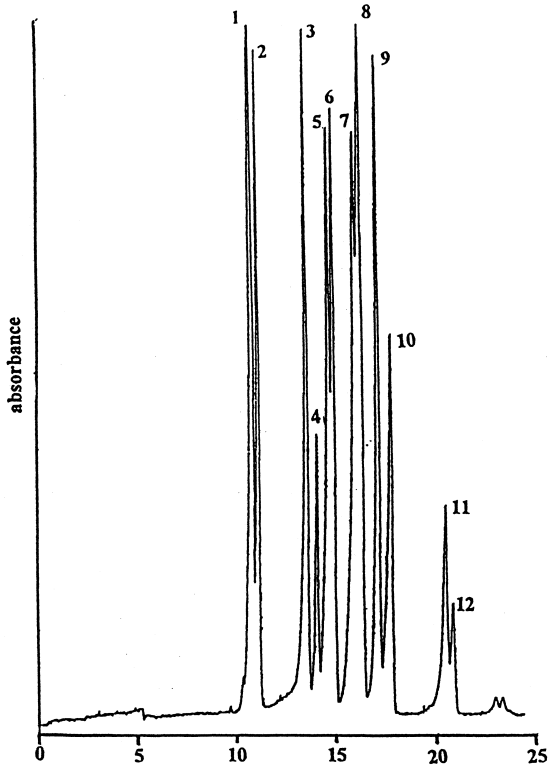

Fig. 13. Electropherogram of a separation of 12 ribonucleotides with stacking (~0.1 μ*M*). The entire capillary was filled with sample (70 cm). Separation conditions: 70 cm×75 μm I.D. column, 60 cm separation distance; 50 m*M* sodium carbonate/bicarbonate buffer, pH 10; −18 kV during stacking, +18 kV for analysis; 254 nm UV absorbance detection. Peaks: 1=AMP, 2=CMP, 3=ADP, 4=GMP, 5=CDP, 6=ATP, 7=UMP, 8=CTP, 9=GDP, 10=GTP, 11=UDP and 12=UTP. Reprinted with permission from Ref. [53].

Whole capillary stacking has been used to increase sensitivity in CE separations of nucleotides. Geldart and Brown [45] used whole capillary stacking to increase the sensitivity of a CZE method for 5′-ribonucleotides. A separation of 12 5′-ribonucleotides with UV detection was performed with a detection limit of 50 n*M*. The nucleotide concentrations in the electropherogram in Fig. 13 are ~5 μ*M*; the detection limit of most UV analyses. Whole capillary stacking has also been applied to CE–UV separations of deoxyribonucleoside monophosphates [16,56], DNA adducts [5,16,40], and nucleoside analogs [6] with a general increase in sensitivity of 200-fold for most analyses. The use of whole capillary stacking has made it possible for the CE–MS characterization of DNA adducts [5,16,40]. Detection limits in the nanomolar range have enabled researchers to identify adducts from DNA hydrosylates. Further research involving in vivo studies is planned [5].

4. CE nucleotide applications

While the development of CE methods for nucleotides has been done primarily with standards, applications to real samples have been performed (Table 2). Problems with compatibility of nucleotide extraction procedures with CE methods was found to be minimal. However, some modifications were necessary for some of the analyses. The matrices varied widely; biological sources included fish [13,43] and guinea pig tissues [34], plasma [31,42] and several types of cells, including oocytes [18], lymphoma [32] and hybridoma [33] cells. In addition, several different DNA adducts were analyzed by UV [16] and identified by CE–MS techniques [5,40]. Other nucleotide sources analyzed with CE include pig feed [35] and fermentation broth [31].

4.1. Tissue and cell samples

Sample preparation for most tissue and cell ma-

Table 2
Nucleotide applications by CE

Matrix	Analytes	Mode	Buffer	pH	Detector	Ref.
Guinea pig organs	5′-Ribonucleotides	CZE	0.02 *M* phosphate 0.5% ethylene glycol	6.36	UV	[34]
Pig feed	IMP, GMP	CZE	20 m*M* sodium borate	9.24	UV	[35]
Fish tissue	Hypoxanthaine, inosine, IMP	CZE	100 m*M* CAPS	11.00	UV	[13]
Fish extract	Hypoxanthine, inosine, IMP	CZE	100 m*M* CAPS	11.00	UV	[43]
DNA hydrosylate	2′-Deoxy 3′-monophosphates	MECC	100 m*M* SDS 5% acetonitrile 20 m*M* sodium phosphate	9.20	UV	[15]
DNA hydrosylate	Deoxymonophosphates DNA adducts	CZE	20 m*M* sodium carbonate	9.60	UV	[16]
DNA hydrosylate	2′-Deoxynucleotides DNA adducts	CZE	100 m*M* ammonium carbonate	9.68	MS	[5]
DNA hydrosylate	dGMP adducts	CZE	10 m*M* ammonium acetate	9.40	MS	[40]
RNA digests (rabbit, calf, yeast)	3′-Monophosphates	MECC	12 m*M* sodium formate 0.1 m*M* CTAB	3.80	UV	[28]
HeLa cells	5′-Ribonucleotides	CZE	50 m*M* Tris–HCl 30 m*M* sodium phosphate	5.30	UV	[2]
Molt 4 human leukemic cells blood lymphocytes	5′-Ribonucleotides	CZE	130 m*M* borate	9.40	UV	[9]
Hydridoma cells	2′-Deoxy-5′-ribonucleotides	CZE	30 m*M* phosphate 50 m*M* Tris–HCl	5.20	UV	[33]
Oocytes	Adenine compounds	CZE	20 m*M* phosphate	8.80	LIF	[18]
Human lymphoma cells	5′-Ribonucleotides	CZE	30 m*M* phosphate 50 m*M* Tris–HCl	5.28	UV	[32]
Cofluent Vero cells	5′-Ribonucleoside triphosphates	MECC	100 m*M* DTAB 1 m*M* EDTA 50 m*M* sodium phosphate	7.00	UV	[8]
Human plasma	Guanosine, 5′-GMP adenine, 5′-AMP	CZE	40 m*M* NaOH		ED	[42]
Plasma	ATP	CZE	50 m*M* ammonium acetate 0.005% HPMC	3.00	UV	[31]
Fermentation broth	Inositol phosphates	CZE	50 m*M* ammonium acetate 0.005% HPMC	3.00	UV	[31]

terial for nucleotide analyses involved nucleotide extraction with either trichloroacetic acid (TCA) [8,13,34,42,43] or perchloric acid [33,34] followed by neutralization with sodium or potassium hydroxide. Methanol has also been used to separate the nucleotides from the remainder of the cell debris [8,9,18]. In a comparison of the two methods, Loregian et al. [8] determined that TCA extraction was better at removing protein contaminants from ribonucleoside triphosphates, while the methanol extraction produced higher quantities of nucleotides. A few researchers filtered the samples [34,43], but the majority of the methods did not cite sample filtration before analysis.

While the sample preparation was relatively simple, many researchers observed matrix effects, including loss of reproducibility, variation of migration time between samples and standards, and stacking effects [9,28,34,42,43]. Reproducibility was improved with the use of an NaOH wash after each run [8,34,35,43]. In most cases, rinsing the capillary returned the reproducibility of the samples to the same level as that achieved with standard nucleotide preparations. Coated capillaries were also used to improve reproducibility by minimization of protein adsorption to the capillary walls [2,32,33]. UCON-coated capillaries were used in the analyses of hybridoma and lymphoma cells [32,33]. Migration time reproducibilities were between 0.2–0.7% for 15 nucleotides and only a buffer rinse between runs was used to prepare the capillary for the next run (Fig. 14).

Differences in migration times between the nucleotides in the sample to those in the standard solutions were caused by either absorption of contaminants in the sample to the capillary wall or by differences in ionic strength of the sample and the standards [2,33,42,43]. Wall interactions were minimized, but not eliminated by the use of coated capillaries [2,32,33] or by rinsing the capillary with NaOH after each run [8,34,35,43]. While these methods were effective in improving reproducibility of the analyses, the selectivity could be different for the nucleotides within the sample when compared to those in a standard solution. Huang et al. [2] used internal standards to spike the sample matrix to determine the migration time of each individual nucleotide in a cell extract. Luong et al. [43] determined that the migration times of hypoxanthine

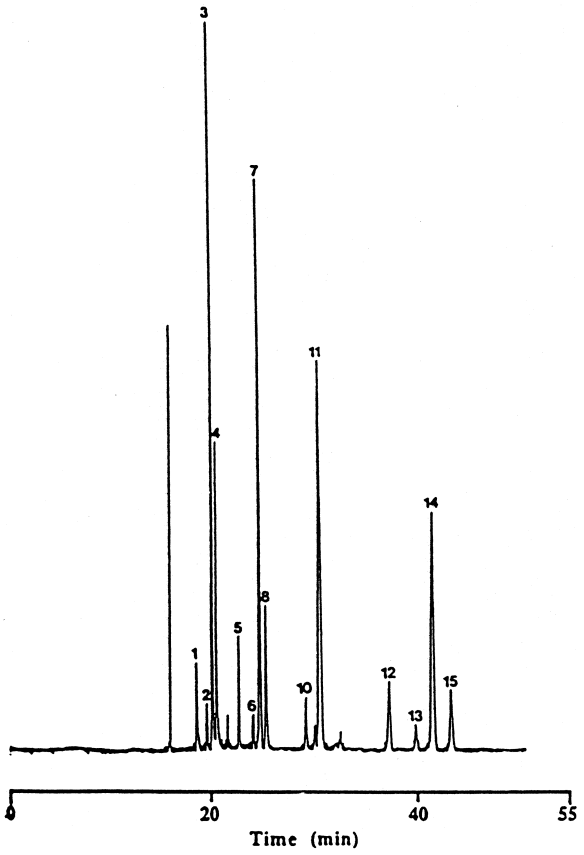

Fig. 14. Electropherogram of ribonucleotides in a hybridoma cell extract. Conditions: Ucon coated capillary, 90 cm×50 μm I.D., 54 cm separation distance; buffer: 30:50 m*M* phosphate–Tris–HCl buffer, pH 5.2; electromigration injection for 6 s at −23 kV; separation voltage: 20 kV. Peaks: 1=UTP, 2=CTP, 3=ATP, 4=GTP, 5=UDP, 6=CDP, 7=ADP, 8=GDP, 9=XMP, 10=UDP-g, 11=ADP-r, 12=UMP, 13=CMP, 14=AMP, 15=GMP. Reprinted with permission from Ref. [33].

compounds in fish tissue varied from those in the standard solution and used internal standards to identify each compound before quantitation.

While protein contaminants from the cell matrix were suspected as being the source of some of the matrix effects, variations in ionic strength between the sample and the standard solutions also affects the resolution and reproducibility of the analysis [33,35]. Several investigators observed higher resolution for the nucleotides in the real samples than for those in the standard nucleotide solution [2,31,22]. The sharper peaks resulted in differences in the migration times between the nucleotides in samples and those

in standard solutions. This difference in resolution between the samples and the standards was found to occur when standards had a higher ionic strength than the cell extract. Thus, the high peak efficiency in the cell extract was due to stacking effects that occur when the difference in ionic strength between the sample and the electrophoretic buffer increase [2,33]. The difference in resolution observed for the nucleotides in standard solutions and sample matrices was eliminated if the ionic strength of the standard solution was reduced [33].

4.2. DNA/RNA sources

While the extraction procedure for nucleotides often resulted in samples with very low ionic strength, the process of enzymatic DNA hydrolysis used by many researchers resulted in a high ionic

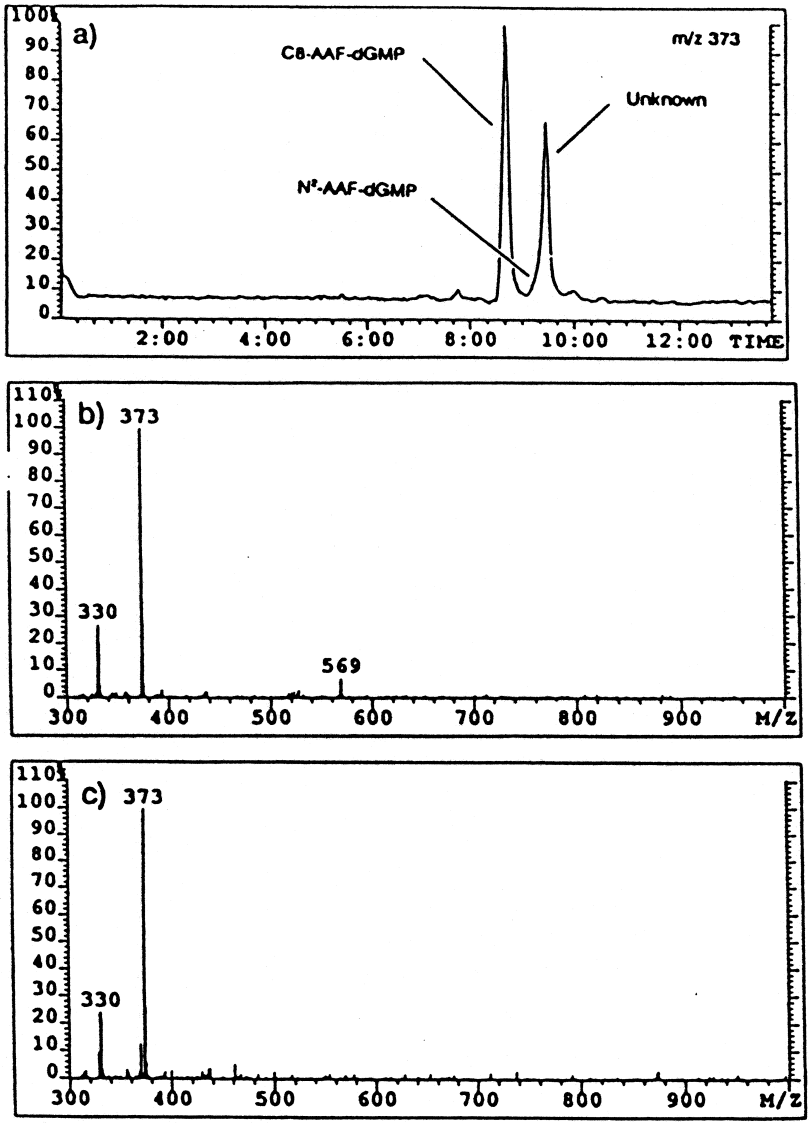

Fig. 15. (a) Mass electropherogram of the *m/z* 373 ion from the CZE–CF-FAB-MS analysis of a 175 nl stacking injection of a fraction of a DNA digest. (b) Full scan FAB mass spectrum of the nucleoside adduct, C8-AAF-dGMP. (c) Full scan FAB spectrum of unknown interference. Reprinted with permission from Ref. [40]. ©1996 American Chemical Society.

strength sample matrix [5,16,40]. Several methods for lowering the salt content of the samples were used. Dilution of the hydrolysis extract reduced the band broadening that resulted from a high ionic strength matrix [5]. However, as the concentration of the DNA adducts in the extract is already low, this method is not always feasible. Solid phase extraction, SPE, [16,40] has been used to remove undigested material, enzymes and non-adducted DNA. In addition it could be used to lower the salt concentration of DNA extracts. Wolf and Vouros [40] used poly(styrene–divinyl benzene) for DNA adduct isolation. No matrix effects were noted after the sample pretreatment. In addition, the use of M_r 3000 cut-off centrifuge filters during centrifugation provided a cleaner sample (Fig. 15).

The concentration of adducted DNA in hydrosylate samples is often very low; one molecule out of 10^{10} molecules is not uncommon [40]. Whole capillary stacking methods have been used to increase the sensitivity of the CE method [5,6,16,40,56], especially if MS is used [5,40]. The use of stacking methods increased the quantity of material available for characterization, and DNA adducts were identified at concentrations below the detection capability of the mass spectrometer. Norwood et al. [16] used CE with UV detection and whole capillary stacking to increase the sensitivity of the method by 200-fold.

4.3. Other nucleotide sources

Sources of nucleotide material include plasma [31,42], fermentation broth [31] and pig feed [35]. Preparation of plasma samples was similar to that for tissue samples. However, dilution was necessary to minimize matrix effects due to the high salt content of the sample matrix [42]. Electrodialysis has been used for the preparation of plasma samples [31]. An on-line electrodialysis–CZE method has been developed for the separation and quantitation of ATP and ITP in human blood plasma. The method involved coupling an electrodialysis device to the CZE system. Samples of fermentation broth and blood plasma were spiked with nucleotides and used to demonstrate that bioanalysis by electrodialysis–CZE is possible. However, more sensitive detection methods are required before analysis of natural nucleotide levels in the biomatrices is possible.

The analysis of IMP and GMP in pig feed by CE was relatively simple [35]. The feed was diluted in water, filtered and injected. Matrix effects due to high salt content of some feeds were eliminated by the addition of equal quantities of NaCl to both samples and standards to equalize the salt content of the feed samples and the standard solutions. A NaOH wash was used to clean the capillary between runs, and reproducibility of the peak area was less than 8%.

5. Conclusions

CE is an attractive alternative to HPLC for the analysis of nucleotides. Since the instrumentation has few moving parts, it is relatively simple and rugged. The analyses are fast and the resolution is high. Plate counts can be greater than 200 000 [17,25]. Only a few nanoliters of sample are required for an analysis. Since the selectivity of the nucleotide separation can be modified by the choice of buffer composition and pH, CE is a flexible technique.

Capillary electrophoresis separations of nucleotides have been performed in both the CZE and MECC modes with a variety of buffers and at different pH levels (Table 2). The parameters of pH, buffer type and mode have been manipulated to obtain the resolution and selectivity, and speed necessary for many different nucleotide analyses. Nucleotide separations can be performed throughout the pH range of 2–11 and are reproducible, sensitive, and selective. Bare capillaries were used for many separations, but coated capillaries could be used to reduce matrix effects or to increase reproducibility. The use of a sodium hydroxide rinse between samples was also used to enhance reproducibility.

A variety of detection methods, including UV, fluorescence, LIF and MS have been used in CE analyses to determine nucleotide concentrations and analog identification.

However, for many nucleotides, especially nucleotides with low extinction coefficients or analytes that must be quantified at concentrations less than 1 μM, the sensitivity of current CE techniques may be inadequate. This limitation occurs because of the relatively high concentration limit of detection of

many detection modes, such as UV and fluorescence, due to the minute sample volume. Preconcentration methods, including solid-phase extraction and stacking methods have been used to increase the concentration sensitivity of CE techniques. One stacking method in particular, whole capillary stacking has increased the sensitivity of CE separation of nucleotides by 200-fold.

Whole capillary stacking has been used in CE–MS and CE–UV analyses, and should enhance the sensitivity of fluorescence and electrochemical methods as well. The advantages of preconcentration by whole capillary stacking include short sample preparation time, minimal expense, and ease of use.

Separations have been performed on nucleotides in a variety of matrices, including plasma, tissues and DNA hydrosylate samples, animal feeds and fermentation broths. CE analyses are usually faster than HPLC analyses and require smaller sample volumes, thus making single cell analyses possible [18].

CE is a valuable technique for the analysis of nucleotides in the pharmaceutical, biochemical, clinical, biotechnology and environmental fields. Further investigations into the separation mechanisms of nucleotides will decrease the time required for method development by enabling the researcher to predict migration behavior before the analysis is performed [46]. The development of sample preconcentration methods, including cITP and stacking methods have expanded the current range of nucleotide analyses below the 1 μM limit to permit low-level detection of nucleotides and analogs. With these developments and additional applications to biological samples, CE will become a practical, inexpensive technique for the routine analysis of nucleotides.

References

[1] S.E. Geldart, P.R. Brown, J. Chromatogr. A 792 (1997) 67–73.
[2] M. Huang, S. Liu, B.K. Murray, M.L. Lee, Anal. Biochem. 207 (1992) 231–235.
[3] J. Cadet, M. Weinfeld, Anal. Chem. 65 (1993) 675A–682A.
[4] W. Li, A. Moussa, R.W. Giese, J. Chromatogr. 608 (1992) 171–174.
[5] D.L.D. Deforce, F.P.K. Ryniers, E. Van den Eekhout, Anal. Chem. 68 (1996) 3575–3584.
[6] S.E. Geldart, P.R. Brown, J. Microcol. Sep. 10 (1998) 65–73.
[7] W.B. Parker, S.C. Shaddix, B.J. Bowdon, L.M. Rose, R. Vince, W.M. Shannon, L.L. Bennett Jr., Antimicrob. Agents Chemother. 37 (1993) 1004–1009.
[8] A. Loregian, C. Scremin, M. Schiavon, A. Marcello, G. Palu, Anal. Chem. 66 (1994) 2981–2984.
[9] M. Ng, T.F. Blaschke, A.A. Arias, R.N. Zare, Anal. Chem. 64 (1992) 1682–1684.
[10] J.S. Walsh, J.E. Patanella, S.E. Unger, K.R. Brouwer, G.T. Miwa, Drug Metab. Dispos. 18 (1990) 1084–1091.
[11] J.E. Patanella, J.S. Walsh, S.E. Unger, G.T. Miwa, P.S. Parry, M.J. Daniel, G.L. Evans, Drug Metab. Dispos. 18 (1990) 1092–1095.
[12] M.W. Roberts, J. Preiss, T.W. Okita, Anal. Biochem. 225 (1995) 121–126.
[13] A.L. Nguyen, J.H.T. Luong, C. Masson, Anal. Chem. 62 (1990) 2490–2493.
[14] S.M. Lunte, D.M. Radzik (Eds.), Pharmaceutical and Biomedical Applications of Capillary Electrophoresis, Elsevier, Amsterdam, 1996, pp. 157–159.
[15] A.F. Lecoq, C. Leuratti, E. Marafante, S. Di Biase, J. High Resolut. Chromatogr. 14 (1991) 667–671.
[16] C.B. Norwood, E. Jackim, S. Cheer, Anal. Biochem. 213 (1993) 194–199.
[17] A.F. Lecoq, S. Di Biase, L. Montanarella, J. Chromatogr. 638 (1993) 363–373.
[18] H.C. Tseng, R. Dadoo, R.N. Zare, Anal. Biochem. 222 (1994) 55–58.
[19] T. Tadey, W.C. Purdey, J. Chromatogr. B 657 (1994) 365–372.
[20] K.H. Row, J.L. Raw, Sep. Sci. Technol. 25 (1990) 323–333.
[21] R. Takigiku, R.E. Schneider, J. Chromatogr. 559 (1991) 247–255.
[22] J.R. Dawson, S.C. Nichols, G.E. Taylor, J. Chromatogr. A 700 (1995) 163–172.
[23] A. Lahey, R.L. St. Claire, III, Am. Lab. Nov. (1990) 68–79.
[24] T. Wang, R.A. Hartwick, P.B. Champlin, J. Chromatogr. 462 (1989) 147–154.
[25] K.H. Row, W.H. Griest, M.P. Maskarinec, J. Chromatogr. 409 (1987) 193–203.
[26] W.H. Griest, P. Maskarinec, K.H. Row, Sep. Sci. Technol. 23 (1988) 1905–1914.
[27] B. Krattiger, A.E. Bruno, H.M. Widmer, R. Dandliker, Anal. Chem. 67 (1995) 124–130.
[28] X. Huang, J.B. Shear, R.N. Zare, Anal. Chem. 62 (1990) 2051–2053.
[29] E. Jackim, C. Norwood, J. High Resolut. Chromatogr. 13 (1990) 195–196.
[30] H. Yamamoto, T. Manabe, T. Okuyama, J. Chromatogr. 480 (1989) 331–338.
[31] B.A.P. Buscher, U.R. Tjaden, J. van de Greef, J. Chromatogr. A 764 (1997) 135–142.
[32] X. Shao, K. O'Neill, Z. Zhao, S. Anderson, A. Malik, M. Lee, J. Chromatogr. 680 (1994) 463–468.
[33] K. O'Neill, X. Shao, Z. Zhao, A. Malik, M.L. Lee, Anal. Biochem. 222 (1994) 185–189.

[34] T. Tsuda, K. Takagi, T. Watanabe, T. Satake, J. High Resolut. Chromatogr., Chromatogr. Commun. 11 (1988) 721–723.
[35] H. Liu, S. Qi, Y. Zhang, A. Huang, S. Yiliang, J. High Resolut. Chromatogr. 20 (1997) 242–244.
[36] M. Uhrova, Z. Deyl, M. Suchanek, J. Chromatogr. B 681 (1995) 99–105.
[37] L. Gross, E.S. Yeung, J. Chromatogr. 480 (1989) 169–178.
[38] W.G. Kuhr, E.S. Yeung, Anal. Chem. 60 (1988) 2642–2646.
[39] R.E. Milofsky, E.S. Yeung, Anal. Chem. 65 (1993) 153–157.
[40] S.M. Wolf, P. Vouros, Anal. Chem. 67 (1995) 891–900.
[41] Z. Zhao, J.H. Wahl, H.R. Udseth, S.A. Hofstadler, A.F. Fuciarelli, R.D. Smith, Electrophoresis 16 (1995) 389–395.
[42] H. Lin, D.-K. Xu, H.Y. Chen, J. Chromatogr. A 760 (1997) 227–233.
[43] J.H.T. Luong, K.B. Male, C. Masson, A.L. Nguyen, J. Food Sci. 57 (1992) 77–81.
[44] S.L. Pentoney, R.N. Zare, J.F. Quint, J. Chromatogr. 480 (1989) 259–27044.
[45] S.E. Geldart, P.R. Brown, Anal. Chem., submitted for publication.
[46] T. Hirakawa, S. Kobayashi, Y. Kiso, J. Chromatogr. 318 (1985) 195–210.
[47] R. Weinberger, Practical Capillary Electrophoresis, Academic Press, San Diego, CA, 1993.
[48] L.A. Holland, N.P. Chetwyn, M.D. Perkins, S.M. Lunte, Pharm. Res. 14 (1997) 372–387.
[49] N.A. Guzman (Ed.), Capillary Electrophoresis Technology (Chromatographic Science Series, Vol. 64) Marcel Dekker, New York, 1993, Ch. 4.
[50] S.F.Y. Li, Capillary Electrophoresis: Principles, Practice and Applications, Elsevier, Amsterdam, 1992, Ch. 5.
[51] J.W. Jorgenson, K.D. Lukacs, Science 222 (1983) 266–272.
[52] B.L. Karger, A.S. Cohen, A. Guttman, J. Chromatogr. 492 (1989) 585–614.
[53] S.E. Geldart, P.R. Brown, Am. Lab. 29 (1997) 48–52.
[54] S. Hjertan, J. Chromatogr. 347 (1985) 191–198.
[55] R.L. Chien, D.S. Burgi, Anal. Chem. 64 (1992) 1046–1050.
[56] J.L. Beckers, M.T. Ackermans, J. Chromatogr. 629 (1993) 371–378.
[57] D.S. Burgi, R.L. Chein, Anal. Biochem. 202 (1992) 306–309.
[58] M. Albin, P.D. Grossman, S.E. Moring, Anal. Chem. 65 (1993) 489A–497A.
[59] P.O.P. Ts'o, Basic Principles in Nucleic Acid Chemistry, Vol. 1, Academic Press, New York, 1974, pp. 461–465.