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Short communication

Separation of purine and pyrimidine bases by capillary zone electrophoresis with carbonate buffers

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Abstract

The separation of eight purine bases was achieved by capillary electrophoresis in 16 min using a voltage of 15 kV and a 50 m*M* sodium carbonate–hydrogencarbonate buffer at a pH of 10. Carbonate buffers have many advantages. They are non-toxic, inexpensive, easy to prepare and provide a stable, reproducible electroosmotic flow. Equilibration time after a sodium hydroxide rinse is minimal; thus the total analysis time is shorter than when an acidic buffer is used. No additives to the buffer are required to obtain the majority of the separations of interest and uncoated capillaries can be used. A group of eight purines and the naturally occurring pyrimidines were baseline separated. The linearity correlation for adenine in an optimized separation of the bases was 0.995 over two orders of magnitude. The daily reproducibility was 0.1% and day-to-day reproducibility was 2.5%.

Keywords: Buffer composition; Purine; Pyrimidines

1. Introduction

The analyses of purine and pyrimidine bases are important in many areas of research. Purine and pyrimidine bases are the building blocks in both DNA and RNA and are involved in cell metabolism [1–4]. Because they are metabolized by the liver, caffeine and some of its metabolites; xanthine, uric acid, theobromine and theophylline have been used to study functional impairment in liver disease [5–8]. Other purine bases, including hypoxanthine have been linked to off-flavors in food and are being investigated as markers for the determination of freshness in food [9,10]. Therefore, quantitative methods for purines and pyrimidines that are quick, inexpensive and accurate are needed. To analyze for purines and pyrimidines, HPLC is currently being used because it is reproducible, sensitive, selective and easily automated [1–5]. However, equipment is fairly expensive, resolution is frequently a problem and a relatively large amount of sample, often up to 100 μl may be required to attain the necessary sensitivity [2,6,9,11,12].

Although purines and pyrimidines are not fully ionized, capillary electrophoresis (CE) can be used to analyze them [2–16]. CE methods, which are fast, inexpensive and use minimal sample, have been developed in both the capillary zone electrophoretic (CZE) [2,3,5,7,9–12] and micellar electrokinetic capillary chromatography (MECC) [4,6,8,13–16] modes. Buffers used to separate bases in the CZE mode include phosphate [2,3], acetate [5], borate [7,11] and CAPS (3-[cyclohexylamino]-1-propane-sulfonic acid) [9,10]. Only a few researchers have reported the use of sodium carbonate in the separation of purines or pyrimidines [7,12]. However, carbonate buffers with a pH range of 8.8–9.8 have been used in CE analyses of nucleotides with great

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success [12,17–21]. Due to the negative charge on purine and pyrimidine bases in this pH range, carbonate buffers seemed to be an alternative to the buffers currently used. Carbonate buffers are non-toxic, inexpensive, easy to prepare, and provide a stable, reproducible electroosmotic flow (EOF) [12,17–20]. When ammonium carbonate is used, the buffer is volatile and it can be readily used with mass spectrometry detection [17]. In addition, carbonate buffers have been used with stacking if better sensitivity is needed [17–19]. Finally, capillary equilibration time after an NaOH rinse is minimal, thus shortening the total analysis time [12,18].

Carbonate buffer has been primarily studied at pH 9.6 [17–19]. However, as the pK_a values of many of the bases are between eight and ten, the buffer pH would have an effect on the selectivity of these compounds. Thus, the effects of voltage, and buffer concentration and pH on the separation purines and pyrimidines were investigated to determine the optimal conditions for the analyses of these compounds using carbonate buffers.

2. Experimental

2.1. Chemicals

Sodium carbonate, sodium hydrogencarbonate, purines and pyrimidines were purchased from Sigma (St. Louis, MO, USA). The purines used were adenine, guanine, theophylline, theobromine, caffeine, xanthine, hypoxanthine and uric acid. The three pyrimidines were thymine, cytosine and uracil. The structures of the bases are shown in Fig. 1. A Milli-Q water purification system (Millipore, Bedford, MA, USA) was used to prepare all samples and buffers.

2.2. Materials

The electrophoresis capillary used was polyimide coated 0.75-μm I.D. purchased from Polymicro Technologies (Phoenix, AZ, USA). Syringe filters (0.45 μm) were used to filter all buffer solutions and base standards. (Fisher Scientific, Eden Prairie, MN, USA).

2.3. Instrumentation

A Waters Quanta 4000 Capillary Electrophoresis unit was used which had a UV detector at 254 nm (Milford, MA, USA) and a 70 cm capillary with the detection window burned in 10 cm from the detector end. Data were collected on a Spectra-Physics integrator Model SP4270 (San Jose, CA, USA).

2.4. Buffer preparation

All buffers were prepared from stock solutions of 100 m*M* sodium carbonate and 100 m*M* sodium hydrogencarbonate. The pH of the sodium carbonate solution was approximately 11.4 and that of the sodium hydrogencarbonate solution was 8.5. Dilutions of each of these buffers were prepared to give concentrations of 10 m*M*, 30 m*M*, and 50 m*M*. The pH levels used in the study were 9, 10, and 11 at each of these concentrations. The desired pH at each concentration was obtained by adding sodium carbonate solution to sodium hydrogencarbonate solution while the solution was monitored with a pH meter. Thus the concentration of the carbonate in the solution was constant even at varying pH values. The solutions were filtered with a 0.45-μm syringe filter before use.

2.5. Standard base preparations

Approximately 5 mg of each base was weighed out and diluted with Milli-Q grade water in volumetric flasks. The resulting concentrations of the bases were between 0.1 and 0.01 mg/ml. The base preparations were filtered with a 0.45-μm syringe filter and stored at 0–5°C. Fresh mixtures of the bases were prepared immediately before analysis and then discarded.

2.6. Capillary preparation

The capillary was cleaned at the beginning of each day by rinsing the capillary according to the following method. The capillary was first rinsed for 5 min with 1 *M* NaOH and then rinsed for 5 min with the buffer to be used in the analysis. After the capillary was filled with the buffer, the CE was run at a

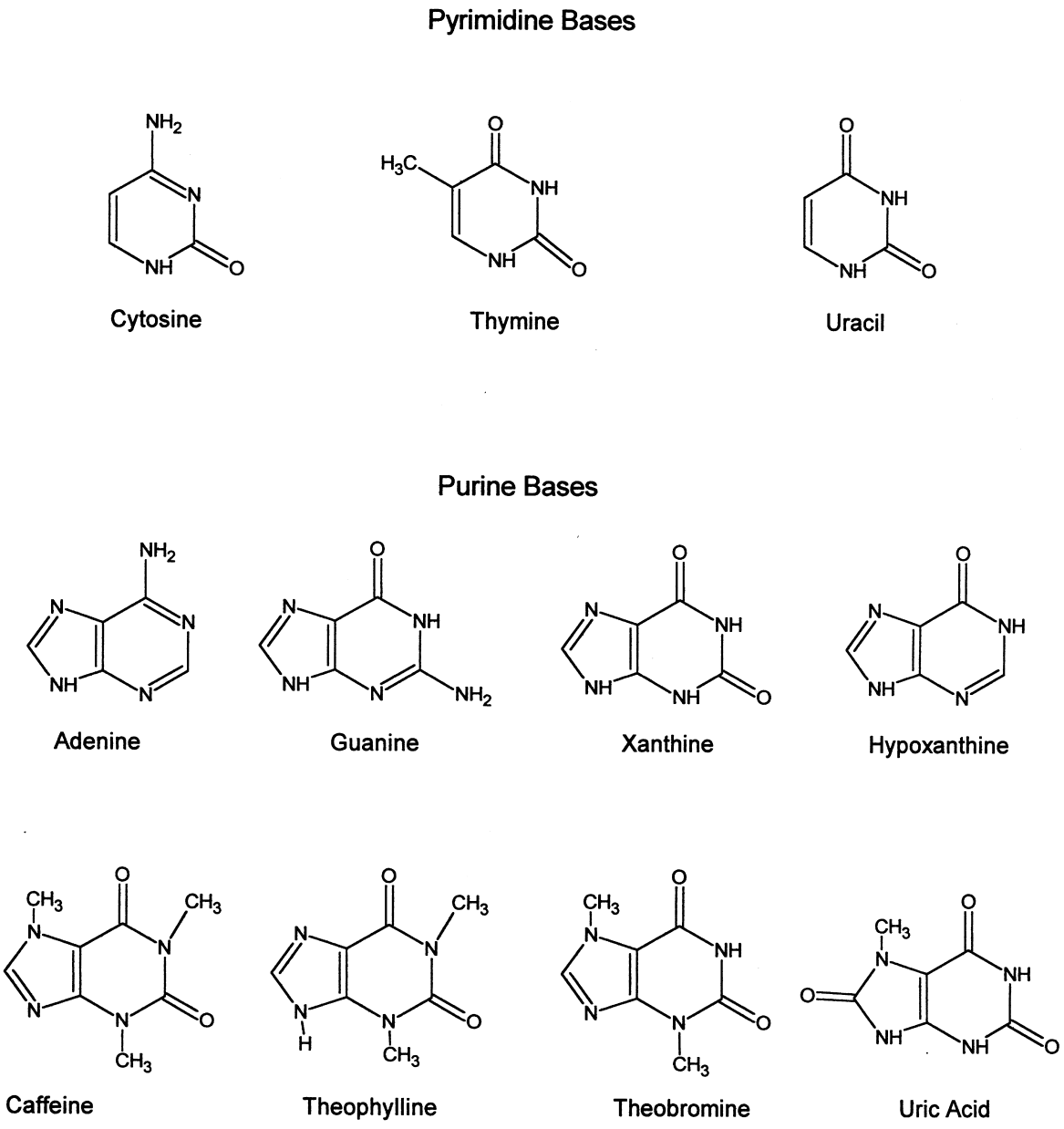

Fig. 1. Structures of pyrimidine and purine bases.

voltage of 15 kV for 30 min to condition the capillary.

2.7. CE method

Preliminary analyses were run with positive polarity and voltages of 12, 15 and 18 kV. Since 15 kV was found to be the best in terms of speed and resolution, this voltage was then used in all experiments. Caffeine was used as a neutral marker to determine the EOF during each set of analyses. The base analyses for each buffer concentration and pH

were run consecutively with a 20-s hydrostatic injection. Concentration and pH ranges were run randomly to avoid any bias that might result from possible column degradation or even conditioning to a certain pH or concentration over time. An integrator was used to collect all data. All data were collected over the course of approximately 6 weeks and the same capillary was used for all runs.

2.8. Method validation

The reproducibility was determined using a carbonate concentration of 50 m*M* and a pH of 10. A solution containing eight purine bases was prepared from the original stock solutions. The resulting concentrations for each base ranged from 2.0 μg/ml to 9.0 μg/ml. Daily reproducibility was calculated for both migration time and peak area for nine consecutive runs a day. The ruggedness of the method was determined by the calculation of day-to-day reproducibility. Nine consecutive runs from a single day and eight from a second day, for a total of 17 runs, were added together to determine the day-to-day reproducibility. The consecutive runs were performed automatically, without washing the capillary between runs or changing the buffer in order to maintain constant volume and ionic strength levels between the inlet and outlet buffer vials. In addition, no correction was made to account for temperature variation; thus any error due to these factors is included in the R.S.D. for the migration times of the purines.

3. Results and discussion

3.1. Voltage and buffer concentration

Voltages between twelve to eighteen kilovolts were investigated for the optimum separation of purine and pyrimidine bases when a buffer concentration of between 30–50 m*M* was used. Fifteen kilovolts was found to provide the best balance between analysis time and resolution.

The buffer range investigated was 10 m*M* to 50 m*M* which is the range most commonly used in CE analyses. As the buffer concentrations increased, the analytes were ‘stacked’ into narrower, sharper peaks because of the large difference in ionic strength between the buffer and the sample. Thus, the peaks were more highly resolved at 50 m*M* concentrations than with a 30 m*M* buffer and had lower detection limits. Below 30 m*M*, the sensitivity and reproducibility of the separation were not acceptable. At buffer concentrations above 50 m*M*, complications from Joule heating were observed in the form of loss of current and decreased reproducibility. While the calculated effective mobilities of the bases were constant within the 30–50 m*M* range, the migration time of the EOF increased substantially with the high concentration buffer. Thus, the analysis time for a separation at 50 m*M* was up to 25% longer than when the same sample was analyzed with a 30 m*M* buffer. Therefore, if speed is of primary importance, the optimal concentration is 30 m*M*, and if high sensitivity, reproducibility and resolution are required, 50 m*M* is best.

3.2. Buffer pH

As can be seen in Table 1, most of the bases have a pK_a in the range of 8–10 [22]. For most separations, pH 10 was found to provide the highest resolution for both the purines and the pyrimidines. Further effects of buffer pH on base mobility for the eight purines and three pyrimidine bases can be seen in Fig. 2a for the three pyrimidines and in Fig. 2b for the eight purines.

Table 1
Ionization constants for purine and pyrimidine bases. All pK_a values are from Ref. [22]

Compound	First pK_a	Second pK_a
Purine bases		
Caffeine	None	
Theobromine	9.97	
Adenine	9.80	
Guanine	9.20	12.20
Hypoxanthine	8.94	12.10
Theophylline	8.81	
Xanthine	7.44	11.12
Uric acid	5.75	10.30
Pyrimidine bases		
Cytosine	12.16	
Thymine	9.94	
Uracil	9.45	

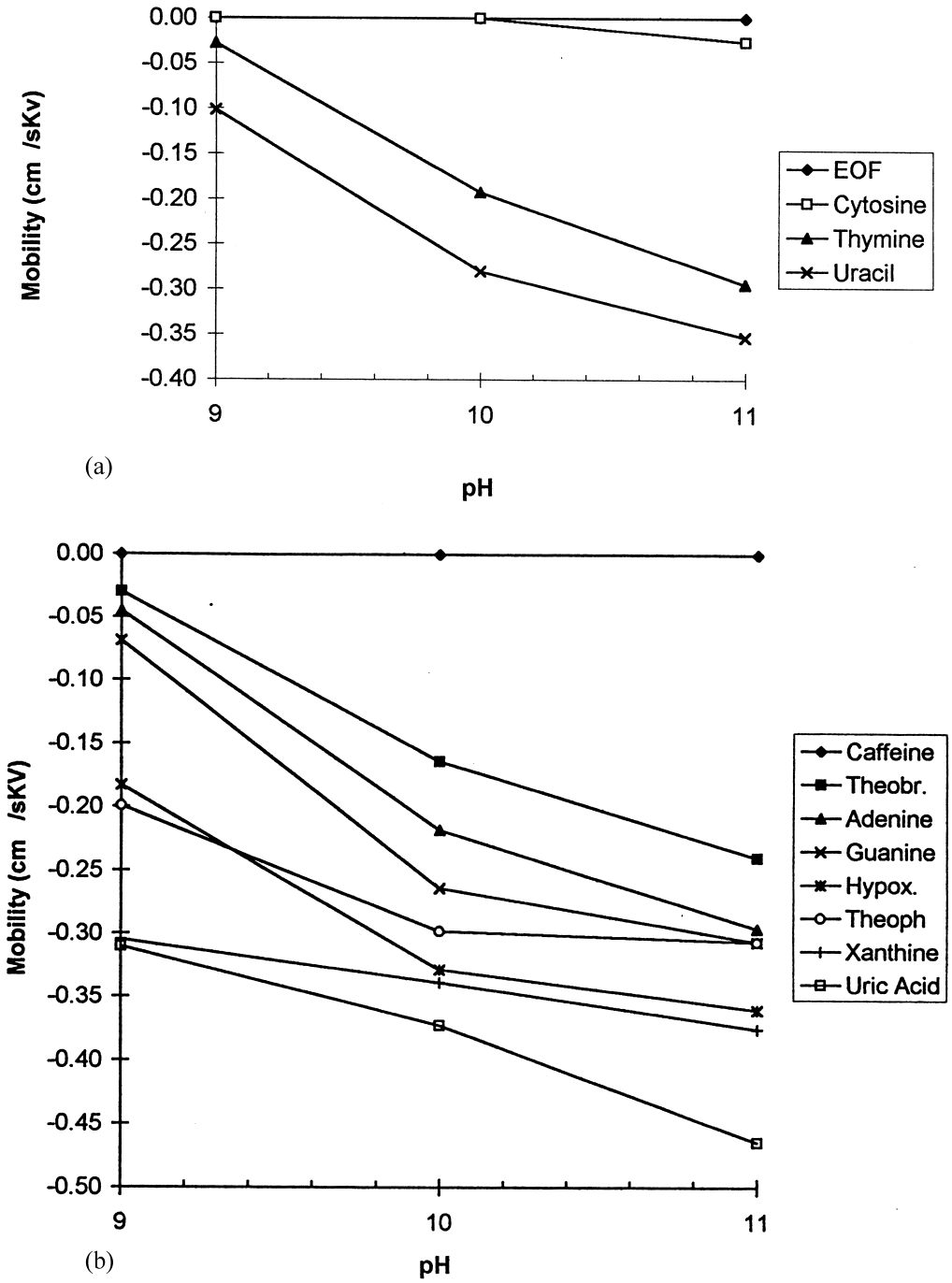

Fig. 2. Effect of pH on pyrimidine and purine mobility. Conditions: buffer, 50 m*M* sodium carbonate–hydrogencarbonate buffer; voltage, 15 kV; hydrodynamic injection for 20 s. Base concentrations from 2 μg/ml–9 μg/ml. (a) Pyrimidines, (b) purines.

3.3. Separation of purine and pyrimidine bases

Baseline separation of the purines was achieved in 16 min and of the pyrimidines in 11 min (Fig. 3a and b). Full separation of all 11 bases was not possible under the experimental conditions investigated due to

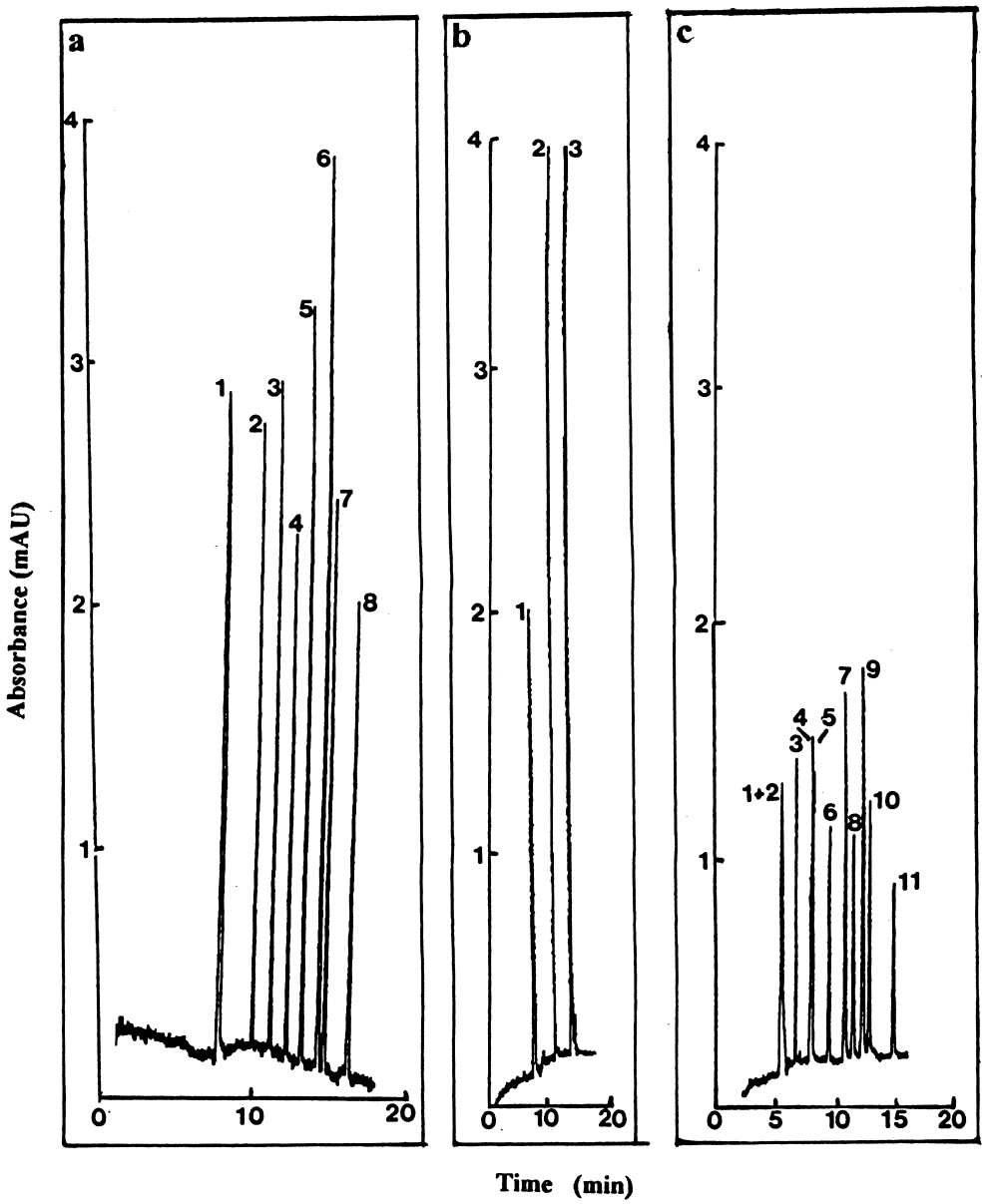

Fig. 3. CE separation of purine and pyrimidine bases. Conditions: buffer, 50 m*M* sodium carbonate–hydrogencarbonate buffer at pH 10; voltage, 15 kV; hydrodynamic injection for 20 s. Base concentrations from 2 μg/ml–9 μg/ml. (a) Purines: 1=caffeine, 2=theobromine, 3=adenine, 4=guanine, 5=theophylline, 6=hypoxanthine, 7=xanthine, 8=uric acid. (b) Pyrimidines: 1=cytosine, 2=thymine, 3=uracil. (c) Mixed bases: 1=caffeine, 2=cytosine, 3=theobromine, 4=adenine, 5=thymine, 6=guanine, 7=theophylline, 8=uracil, 9=hypoxanthine, 10=xanthine, 11=uric acid.

the migration of cytosine and caffeine with the EOF and the comigration of adenine and thymine (Fig. 3c).

3.4. Method validation

The method was both reproducible and rugged. Daily reproducibility was between 0.1% and 0.2% for migration time and 1% and 2% for peak area. Day-to-day reproducibility ranged from 1.4% to 2.2% for migration time and from 0.8% to 4.6% for peak area.

Adenine was used as a model compound to calculate the linear range. A stock solution of 26 μg/ml of adenine was diluted with Milli-Q water and analyzed. Additional dilutions were made from the stock solution and tested until the detection limit was achieved. The detection limit was 0.02 μg/ml. The linear range extended from 26 μg/ml to 0.04 μg/ml with a correlation coefficient of 0.995.

4. Conclusions

Purine and pyrimidine base separations by CE with a sodium carbonate buffer are reproducible, sensitive, and selective. Sodium carbonate–hydrocarbonate buffers are inexpensive and easy to prepare and store. Since the equilibration time after washing the capillaries with NaOH was minimal, the total analysis time was fast. The capillaries were inexpensive and required no treatment other than an occasional wash with NaOH to restore the inner surface. No buffer additives were needed to attain adequate resolution for the majority of the analyses performed. In addition, a volatile carbonate buffer, such as ammonium carbonate can be readily used when the CE is interfaced with mass spectrometry detection [17]. Thus, CE with a carbonate buffer is a practical alternative to other buffers for CE analyses of purine and pyrimidine bases.

References

[1] J. Cadet, M. Weinfeld, Anal. Chem. 65 (1993) 675–682A.
[2] H.C. Tseng, R. Dadoo, R.N. Zare, Anal. Biochem. 222 (1994) 55–58.
[3] H. Lin, D.-K. Xu, H.Y. Chen, J. Chromatogr. A 760 (1997) 227–233.
[4] A.F. Lecoq, C. Leuratti, E. Marafante, S. Di Biase, J. High Resolut. Chromatogr. 14 (1991) 667–671.
[5] N. Rodopoulos, A. Norman, Scand. J. Clin. Lab. Invest. 54 (1994) 305–315.
[6] Y. Zhao, C.E. Lunte, J. Chromatogr. B 688 (1997) 265–274.
[7] Z.K. Shihabi, M.E. Hinsdale, A.J. Bleyer, J. Chromatogr. B 669 (1995) 163–169.
[8] I.Zk. Atamna, G.M. Janini, G.M. Muschik, H.J. Issaq, J. Liq. Chromatogr. 14(3) (1991) 427–435.
[9] A.L. Nguyen, J.H.T. Luong, C. Masson, Anal. Chem. 62 (1990) 2490–2493.
[10] J.H.T. Luong, K.B. Male, C. Masson, A.L. Nguyen, J. Food Sci. 57(1) (1992) 77–82.
[11] T. Grune, G.A. Ross, H. Schmidt, W. Siems, D. Perrett, J. Chromatogr. 636 (1993) 101–111.
[12] S.E. Geldart, P.R. Brown, Anal. Chem. (submitted).
[13] A.S. Cohen, S. Terabe, J.A. Smith, B.L. Karger, Anal. Chem. 59 (1987) 1021–1027.
[14] A.S. Cohen, A. Paulus, B.L. Karger, Chromatographia 24 (1987) 15–23.
[15] D.E. Burton, M.J. Sepaniak, M.P. Maskarinec, Chromatographia 21 (1986) 583–586.
[16] M. Korman, J. Vindevogel, P. Sandra, Electrophoresis 15 (1994) 1304–1309.
[17] D.L.D. Deforce, F.P.K. Ryniers, E. Van den Eekhout, Anal. Chem. 68 (1996) 3575–3584.
[18] S.E. Geldart, P.R. Brown, J. Microcol. Sep. 10 (1998) 65–73.
[19] C.B. Norwood, E. Jackim, S. Cheer, Anal. Biochem. 213 (1993) 194–199.
[20] S.E. Geldart, P.R. Brown, J. Chromatogr. A 792 (1998) 62–73.
[21] S.E. Geldart, P.R. Brown, Am. Lab. 29 (1997) 48–52.
[22] H.A. Sober (Ed.), Handbook of Biochemistry, 2nd ed., Chemical Rubber Co., Cleveland, OH, 1970.