Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Modification of the Capillary Zone Electrophoretic Retention Behavior of Deoxyribonucleotides by Different Mobile Phases

K. H. Row[a]; J. I. Raw[b]
[a] SEPARATION PROCESS LABORATORY DIVISION OF CHEMICAL ENGINEERING, KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY, SEOUL, KOREA [b] COLLEGE OF ENGINEERING INHA UNIVERSITY, INCHEON, KOREA

To cite this Article Row, K. H. and Raw, J. I.(1990) 'Modification of the Capillary Zone Electrophoretic Retention Behavior of Deoxyribonucleotides by Different Mobile Phases', Separation Science and Technology, 25: 3, 323 — 333
To link to this Article: DOI: 10.1080/01496399008050335
URL: http://dx.doi.org/10.1080/01496399008050335

SEPARATION SCIENCE AND TECHNOLOGY, **25**(3), pp. 323-333, 1990

Modification of the Capillary Zone Electrophoretic Retention Behavior of Deoxyribonucleotides by Different Mobile Phases

K. H. ROW*

SEPARATION PROCESS LABORATORY
DIVISION OF CHEMICAL ENGINEERING
KOREA INSTITUTE OF SCIENCE AND TECHNOLOGY
P.O. BOX 131, CHEONGRYANG, SEOUL, KOREA

J. I. RAW

COLLEGE OF ENGINEERING
INHA UNIVERSITY
253 YONGHYEON-DONG, NAM-KU, INCHEON, KOREA

Abstract

The effect of the addition of isopropanol as a organic modifier in capillary zone electrophoresis is discussed in terms of resolution using the four deoxyribonucleotides of dAmp, dGmp, dTmp, and dCmp. The system consisted of 60-μm i.d. fused silica tubing and 68.5 cm column length, a high voltage power supply, and a UV detector set at 254 nm. The effect of the eight different mobile phases on the column efficiency and resolution were compared to find the optimum mobile phase. The experimental results showed that the mobile phase of phosphate/Tris with 0.05 *M* SDS was most suitable for the separation of the components, and that the column efficiency and resolution were affected by the separation voltage.

INTRODUCTION

Capillary zone electrophoresis (CZE) had developed into a powerful technique for the separation and analysis of charged substances, es-

*To whom correspondence should be addressed.

pecially biopolymers (*1, 2*). Contrary to polyacrylamide or cellulose acetate membranes, an aqueous system is advantageous in the dissipation of heat generated by the application of high voltage. Microbore tubing with a thin wall is most favorable to avoid a broadening of the sample band (*3*). Samples are migrated based on electroosmosis and electrophoresis.

It is possible that the use of a mobile phase modifier to extend the elution range would improve the separation as reported for uncharged solutes by Balchunas and Sepaniak (*4*). That is, the use of organic or aqueous organic media may extend the applicability of CZE to a wider range of compounds having low solubility in water. In CZE, differences in the viscous drag of neutral solutes, primarily as a result of size differences, can provide for their separation. However, these differecnes, are usually very small and, consequently, the technique is not very useful for separating neutral compounds. With micellar electrokinetic capillary chromatography (MECC), a surfactant is added to the mobile phase at a concentration above its critical micelle concentration. The resulting micelles provide an effective mechanism for separating neutral compounds which are separated based on their differential partitioning between a mobile phase and the hydrophobic interior of the micelles (*5, 6*).

In this work, eight different mobile phases are used for the separation of four deoxyribonucleotides. The organic modifier of isopropanol or acetonitrile and/or the surfactant of sodium dodecyl sulfate are added in the mobile phases to compare the number of theoretical plates and resolution with separation voltages.

EXPERIMENTAL

Reagents and Apparatus

The deoxyribonucleotides used in this study and their abbreviations are 2′-deoxyadenosine-5′-monophosphate (dAmp), 2′-deoxyguanosine-5′-monophosphate (dGmp), 2′-deoxycytidine-5′-monophosphate (dCmp), and 2′-deoxythymidine-5′-monophosphate (dTmp). The compounds were obtained from the Pharmicia Chemical Co. (Piscataway, New Jersey). Sodium dodecylsulfate (SDS), Tris, and the buffer components (sodium tetraborate and sodium phosphate) were purchased from the Aldrich Chemical Co. (Milwaukee, Wisconsin). The column was 60 μm i.d. fused silica tubing from SGE, Inc. (Austin, Texas). The effective column length to the UV absorbance detector was 68.5 cm. The samples were purchased from the Aldrich Chemical Co. The high voltage power

supply, provided by Hipotronics (Brewster, New York), could be extended to 40 kV. The two ends of the capillary column were put into two separate reservoirs, and the surface of a small section of the column was removed for a UV detector from JASCO (UVIDEC-100-III Spectrophotometric detector, Japan Spectroscopic Co.). Its signals at 254 nm were continuously displayed on a strip chart recorder. Duplicate Plexiglas boxes protected the operator from contact with high voltage.

Methods

A new column was cleaned by rinsing with 0.1 *M* HCl for several hours or overnight, and then for 1 h with deionized water and the mobile phase. The sample was introduced on-column by using electroinjection (*7, 8*). The column was rinsed frequently between runs with the mobile phase to maintain reproducibility in electric current and retention times. The number of theoretical plates N for each component i was calculated from the peak width at the baseline (W_i, in seconds) and retention time (T_{ri}, in seconds) from

$$N = 16(T_{ri}/W_i)^2 \quad (1)$$

The resolution of the peaks for two sequentially eluting components i and j was calculated from their peak widths and retention times from

$$R_{ij} = 2(T_{rj} - T_{ri})/(W_j + W_i) \quad (2)$$

RESULTS AND DISCUSSION

The diversity of available mobile phases enhances any separation technique. The choice of the proper organic modifier or surfactant may be critical to attaining the desired separation or adjusting the retention time and column efficiency. Figure 1 shows the effect of the organic modifier of isopropanol on the resolution at the separation voltage of 38 kV. Without the isopropanol in borate/phosphate buffer, only the dAmp and dGmp were resolved. By increasing the isopropanol concentration to about 1%, the resolutions were improved, while those between dAmp and dGmp kept increasing even above 1%. This may be attributed to lower electrophoretic mobility of the pyrimidine-containing species by viscous drag. At 38 kV the efficiency for dAmp in borate/phosphate buffer is 23,800

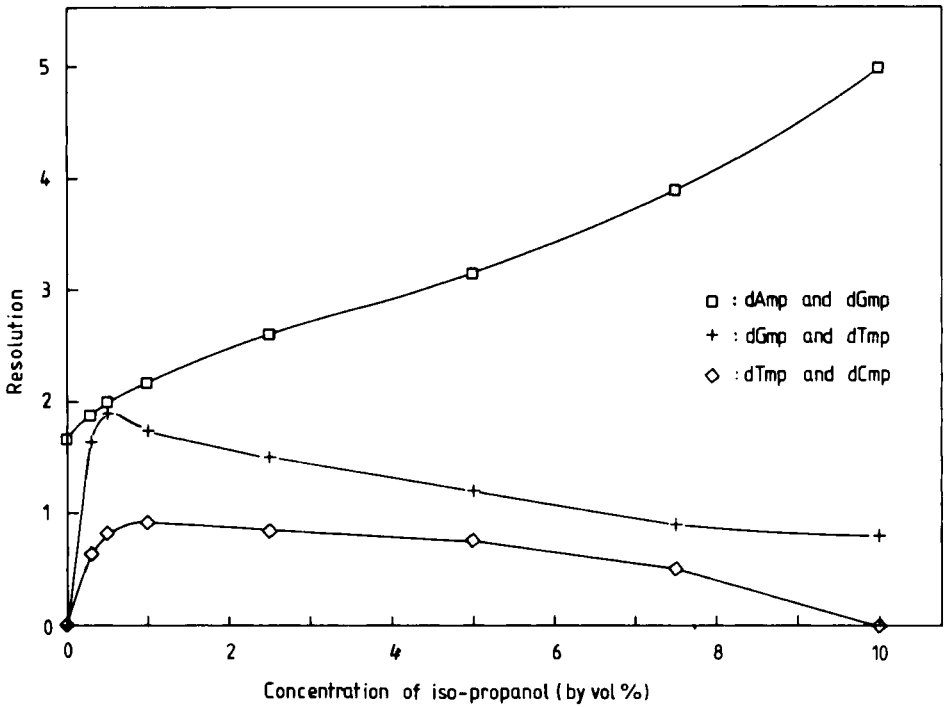


FIG. 1. Relationship between concentration of isopropanol and resolution (0.01 *M* Na_2HPO_4, 0.006 *M* $Na_2B_4O_7 \cdot 10\ H_2O$, 38 kV).

theoretical plates, but the addition of 1% isopropanol gives 34,700 plates (see Table 3). It was observed that the retention time was not changed much below 1% isopropanol. It is interesting that a small portion of the organic modifier provides better resolution, mainly due to the narrower peak width. Above 1% isopropanol, the electric current decreased and the retention time increased. This means that more added isopropanol tended to tighten the components, so that the local viscosity was increased, thereby decreasing the mass transfer kinetics.

For all the mobile phases tested in this work, the relative elution order was not changed: dAmp < dGmp < dTmp < dCmp. The relative order of pyrimidines before purines was observed for nucleic bases and nucleosides (*9*). This is similar to the order of retention by reversed-phase high performance liquid chromatography (*10*). The greater retention of the purines over the pyrimidines suggests their greater solubility in the mobile

phase. Therefore, dAmp and dGmp elute earlier than dTmp and dCmp. This may be a result of the slightly more bulky nature of the purine groups (two aromatic rings) versus the pyrimidine groups (one aromatic ring). The acidic phosphate group in the nucleotides is ionized, imparting a double negative charge to the molecule.

The mobile phases used in this work are listed in Table 1. As a buffer solution, two of sodium phosphate, sodium borate, and trishydroxymethylaminomethane were mixed. One of isopropanol, acetonitrile, and urea was used as a organic modifier, with sodium dodecyl sulfate as a surfactant. The effect of the mobile phases on retention time with the different separation voltages is shown in Table 2. The linear velocity of eluent flow through a column is determined by the electric field strength, which is much greater than the electrophoretic flow. Therefore, at a higher separtion voltage, the retention time is decreased. The phosphate/borate buffer (#1) gave the shortest retention time at a given voltage. Adding a organic modifier and/or a surfactant of SDS to the mobile phase increases the retention time up to 200%.

The column efficiency is highly dependent upon the mobile phases used. Table 3 shows the dependence of the mobile phase on the number of theoretical plates. Only in the mobile phases of #4 and #5 were the number of theoretical plates attained for the four components at the three separation voltages. For some species more than 1,167,000 theoretical plates (170,000 theoretical plates/m) are realized. Generally, at a given mobile phase the column efficiency decreases as the separation voltage increases from 15 to 38 kV.

TABLE 1
Types of Mobile Phase Used in This Work[a]

Mobile phase	Na_2HPO_4	$Na_2B_4O_7 \cdot 10\ H_2O$	Tris	Organic modifier	SDS
1	0.01	0.006			
2	0.01	0.006		Isopropanol, 1.0%[b]	
3	0.01	0.006		Acetonitrile, 5.0%[b]	
4	0.005		0.02		
5	0.005		0.02		0.05
6	0.005	0.02		Isopropanol, 0.5%[b]	0.05
7	0.005		0.02	7 *M* Urea	0.05
8	0.01	0.006			0.05

[a]Units = *M*; injection condition: 5 s, 5 kV.

[b]By volume.

TABLE 2
Effect of Mobile Phases on Retention Time

Mobile phase	Separation voltage (kV)	Retention time (min)			
		dAmp	dGmp	dTmp	dCmp
1	15	16.8	[a]	[a]	[a]
2		20.2	21.2	[a]	[a]
3		20.5	21.0	[a]	[a]
4		17.2	17.9	24.1	24.3
5		20.7	21.9	30.7	32.2
6		18.5	19.4	[a]	[a]
7		19.4	20.2	[a]	[a]
8		21.4	[a]	[a]	[a]
1	30	7.1	7.4	[a]	[a]
2		7.6	7.9	8.1	8.2
3		7.9	8.3	8.5	8.6
4		7.5	7.9	11.2	11.6
5		7.2	7.5	8.6	8.8
6		7.2	7.5	8.1	8.3
7		9.2	9.6	10.8	11.0
8		7.5	8.0	[a]	[a]
1	38	4.6	4.8	[a]	[a]
2		5.1	5.4	5.5	5.6
3		5.5	5.6	5.8	6.0
4		5.3	5.6	8.0	8.3
5		4.8	5.1	5.5	5.7
6		4.7	4.9	5.1	5.2
7		7.1	7.5	8.4	8.8
8		4.0	4.2	4.3	4.4

[a]Coeluting peaks.

TABLE 3
Effect of Mobile Phases on Number of Theoretical Plates

Mobile phase	Separation voltage (kV)	N_{dAmp}	N_{dGmp}	N_{dTmp}	N_{dCmp}
1	15	36,900	[a]	[a]	[a]
2		99,200	104,600	[a]	[a]
3		86,300	76,100	[a]	[a]
4		59,600	54,300	37,100	50,600
5		59,500	31,900	22,600	25,100
6		60,600	37,500	[a]	[a]
7		37,500	26,000	[a]	[a]
8		116,700	[a]	[a]	[a]
1	30	26,200	29,800	[a]	[a]
2		91,300	69,400	97,000	33,000
3		53,500	46,300	11,700	31,000
4		41,900	44,500	8,000	36,100
5		32,900	31,000	29,900	45,400
6		57,600	28,100	36,700	55,100
7		25,500	22,500	23,500	33,700
8		90,000	52,300	[a]	[a]
1	38	23,800	25,900	[a]	[a]
2		34,700	27,500	76,200	15,800
3		48,100	35,300	67,200	33,600
4		30,700	12,400	6,400	27,500
5		26,700	30,800	21,900	43,700
6		35,100	25,600	30,900	43,500
7		20,200	10,000	9,200	31,000
8		15,100	9,800	29,600	31,000

[a]Coeluting peaks.

TABLE 4
Effect of Mobile Phases on Resolution[a]

Mobile phase	Separation voltage (kV)	R_{1-2}	R_{2-3}	R_{3-4}
1	15	[b]	[b]	[b]
2		3.64	[b]	[b]
3		2.71	[b]	[b]
4		1.40	11.18	0.64
5		4.80	13.50	2.00
6		2.57	[b]	[b]
7		1.68	[b]	[b]
8		[b]	[b]	[b]
1	30	1.27	[b]	[b]
2		3.18	1.71	0.67
3		3.17	2.27	0.37
4		1.63	7.76	0.91
5		2.69	6.29	1.11
6		1.63	4.00	0.80
7		1.60	3.57	0.62
8		0.47	[b]	[b]
1	38	1.67	[b]	[b]
2		2.17	1.67	0.92
3		1.36	1.90	0.91
4		1.88	8.00	1.10
5		2.30	1.76	1.54
6		1.20	1.50	1.00
7		1.60	2.07	0.95
8		1.33	0.74	0.50

[a] R_{1-2} = resolution between dAmp and dGmp. R_{2-3} = resolution between dGmp and dTmp. R_{3-4} = resolution between dTmp and dCmp.

[b] Coeluting peaks.

The influences of the mobile phases upon the resolution of neighboring pairs of the four mononucleotides are illustrated by the data in Table 4. In the mobile phases of #4 and #5 which contain Tris and #7 which does not, the four components are resolved at separation voltages of 15, 30, and 38 kV. Tris has been a major biochemical buffer for many years because it is relatively inexpensive and is readily available in a highly purified form (*11*). Urea was included in the mobile phase to disrupt association and improve efficiency in the MECC separation of ribonucleotide octamers (*12*). Comparing mobile phase #7 to #4 and #5 shows that urea does not contribute to better resolution. This means that as the urea is contained, the mononucleotides in #4 associate with it, and those in #5 are not effectively distributed by their different partition equilibriums between the micelle and the phosphate/Tris aqueous phase. Based on the resolution reviewed in the table, the mobile phase of #5 is a desirable condition. In the mobile phase of SDS and phosphate/borate buffer (#8), the four mononucleotides were not completely separated at the lower voltage of 15kV, and the dAmp was only resolved from the other three mononucleotides. The micelles of SDS are negatively charged and move at a velocity which is much lower than the electroosmotic flow and opposite in direction due to electrophoretic effects. The higher separation voltage at 38 kV can render slightly better resolution. It seems that because of their negative charge, the nucleotides are not expected to partition effectively into the SDS micelles, which also are negatively charged on their exterior. However, by using 0.075 *M* SDS (*9*), the column efficiency and resolution were greatly improved, which indicates that a higher SDS concentration decreases the band broadening due to resistance to mass transfer in the mobile phase by effectively decreasing the intermicelle particle distance.

Figure 2 shows the separations of the four mononucleotides in three different experimental conditions. Comparison of Figs. 2(a) and 2(b) shows that the mobile phases are different [phosphate/borate buffer (a) and phosphate/Tris buffer with 0.05 *M* SDS (b)], but the same separation voltage of 15 kV is applied. By using the mobile phase #5, the unresolved peak of dGmp, dTmp, and dCmp is separated, while the retention times are increased. The longer retention times can be compensated for by increasing the separation voltage to 38 kV (c), where the components are completely resolved in less than 6 min.

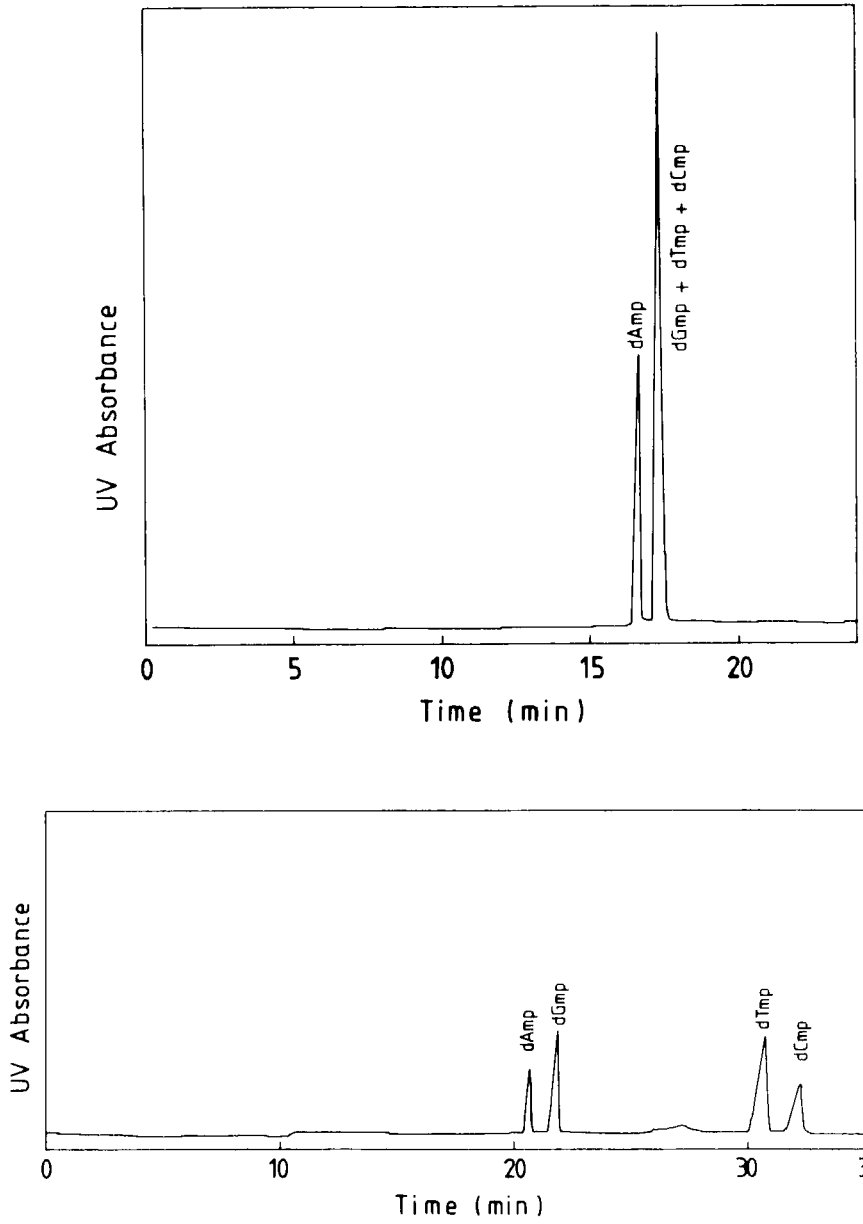
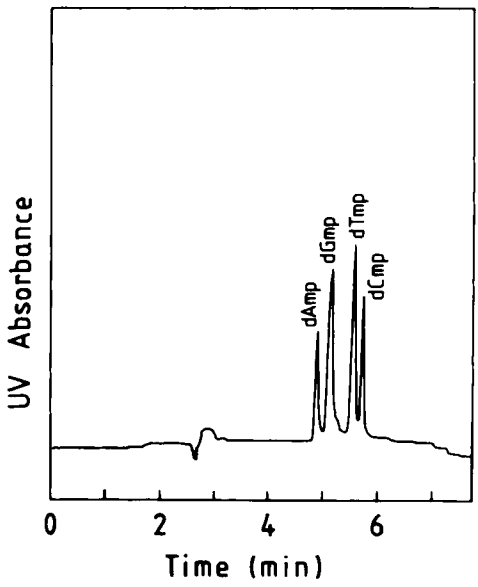


FIG. 2. Electrophoretic separations of mononucleotides. Type of mobile phase: #1 (a) 15 kV; #5 (b) 15 kV, (c) 38 kV.



Acknowledgment

The experimental work was performed at Oak Ridge National Laboratory, Tennessee, USA.

REFERENCES

1. H. H. Lauer and D. McManigill, *Anal. Chem., 58,* 166 (1986).
2. H. H. Lauer and D. McManigill, *Trend Anal. Chem., 5,* 11 (1986).
3. J. W. Jorgenson and K. D. Lukacs, *Anal. Chem., 53,* 1298 (1981).
4. A. T. Balchunas and M. J. Sepaniak, *Ibid., 59,* 1466 (1987).
5. S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, and T. Ando, *Ibid., 56,* 111 (1984).
6. D. W. Armstrong, *Sep. Purif. Methods, 14,* 213 (1985).
7. D. E. Burton, M. J. Sepaniak, and M. P. Maskarinec, *Chromatographia, 21,* 583 (1986).
8. D. E. Burton, M. J. Sepaniak, and M. P. Maskarinec, *J. Chromatogr. Sci., 24,* 347 (1986).
9. K. H. Row, W. H. Griest, and M. P. Maskarinec, *J. Chromatogr., 409,* 193 (1987).
10. C. W. Gehrke, R. A. McCune, M. A. Gama-Sosa, M. Ehrlich, and K. C. Kuo, *Ibid., 301,* 199 (1984).
11. D. D. Perrin and B. Dempsey, *Buffers for pH and Metal Ion Control,* Wiley, New York, 1974.
12. A. S. Cohen, S. Terabe, J. A. Smith, and B. L. Karger, *Anal. Chem., 59,* 1021 (1987).

Received by editor March 20, 1989