ELSEVIER

Journal of Chromatography A, 853 (1999) 349–354

JOURNAL OF CHROMATOGRAPHY A

Short communication

Capillary electrophoresis of double-stranded DNA in an untreated capillary

Zak K. Shihabi*

Pathology Department, Wake Forest University Baptist Medical Center, Winston-Salem, NC 27157, USA

Abstract

Using the zwitterionic buffer *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid (HEPES) in the presence of a high-molecular-mass hydroxypropylmethylcellulose (HPMC) as a sieving polymer and ethidium bromide double-stranded DNA (dsDNA) was separated in an untreated capillary. The HEPES buffer shielded the DNA against the capillary wall interaction and decreased the electroosmotic flow enabling a good separation of the DNA similar to that obtained in a commercially coated capillary. In addition to the low cost of the untreated capillary it can be washed after each run. Furthermore, stacking with hydrodynamic injection filling about half of the capillary volume is demonstrated.

Keywords: Sample stacking; Buffer composition; DNA

1. Introduction

Capillary electrophoresis (CE) offers speed and ease to the analysis of the double-stranded DNA (dsDNA), especially the polymerase chain reaction (PCR) products. dsDNA separations usually are performed in coated capillaries to overcome the wall interactions and eliminate the effects of the electroosmotic flow (EOF). Capillaries with different coatings are used in this respect [1–4]. Commercially coated capillaries are expensive. In addition to this they can have very short lives. Previously, untreated capillaries used with hydroxyethylcellulose (HEC) gave reversed separations in which the large size fragments migrated first due to the action of the EOF [5]. Gao and Yeung [6] have described DNA separations in non-coated capillaries using poly(vinylpyrrolidone) which gave separations similar to that of the coated capillaries; however, such capillaries can be used for about 30 runs. Fang and Yeung [7] also described separation of high speed DNA sequencing with bare capillaries in the presence of poly(ethylene oxide) in which the capillary is rinsed with 0.1 *M* HCl for 15–30 min between injections in order to retitrate the silanol groups to their protonated state.

Tris(hydroxymethyl)aminomethane (Tris)–borate buffer with the addition of a polymer has been utilized in most of the DNA separations [1–7]. Polymers enable the separation based on size rather than charge to mass ratio. Different polymers such as hydroxypropylmethylcellulose (HPMC) and HEC have been successfully used. The optimum concentration and the molecular mass are more important than the type of the polymer [8]. HPMC in coated capillaries and in Tris buffer has been used in many DNA separations [1–4]. Here we show that when HPMC, as a sieving agent, is added to *N*-2-hydroxyethylpiperazine-*N′*-2-ethanesulfonic acid

*Tel.: +1-910-716-2639; fax: +1-910-716-9944.
E-mail address: zshihabi@wfubmc.edu (Z.K. Shihabi)

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00485-9

(HEPES), a zwitterionic buffer, the separation can be accomplished in a non-coated capillary for over 100 injections. Zwitterionic buffers have been used previously to shield proteins [9], peptides [10,11], and basic drugs [12] against capillary wall interactions. Furthermore, we show that dsDNA can be stacked in these capillaries by hydrodynamic injection after dissolving the sample in a weak phosphate buffer. The stacking is very important to improve the sensitivity when detection at 254 nm is used.

2. Experimental

2.1. CE instrument

A Model 2000 CE instrument (Beckman Instruments, Fullerton, CA, USA) was set at 200 V/cm (reversed polarity) and at 254 nm. Untreated silica capillary 25 cm (effective length 17.5 cm)×75 μm (I.D.) was washed initially for 3 min with 0.2 *M* NaOH and for 3 min with 0.2 *M* HCl and finally filled for 3 min with the separation buffer. The sample was injected for 99 s, at low pressure, filling about 25% of the capillary volume and electrophoresed for 10 min. The capillary is washed between samples with 0.2 *M* NaOH for 0.5 min and with 0.2 *M* HCl for 0.5 min. Finally it was filled with the DNA separation buffer for 2 min.

2.2. DNA buffer

The separation buffer contained the following at the final concentration per liter (pH 8.1): HEPES

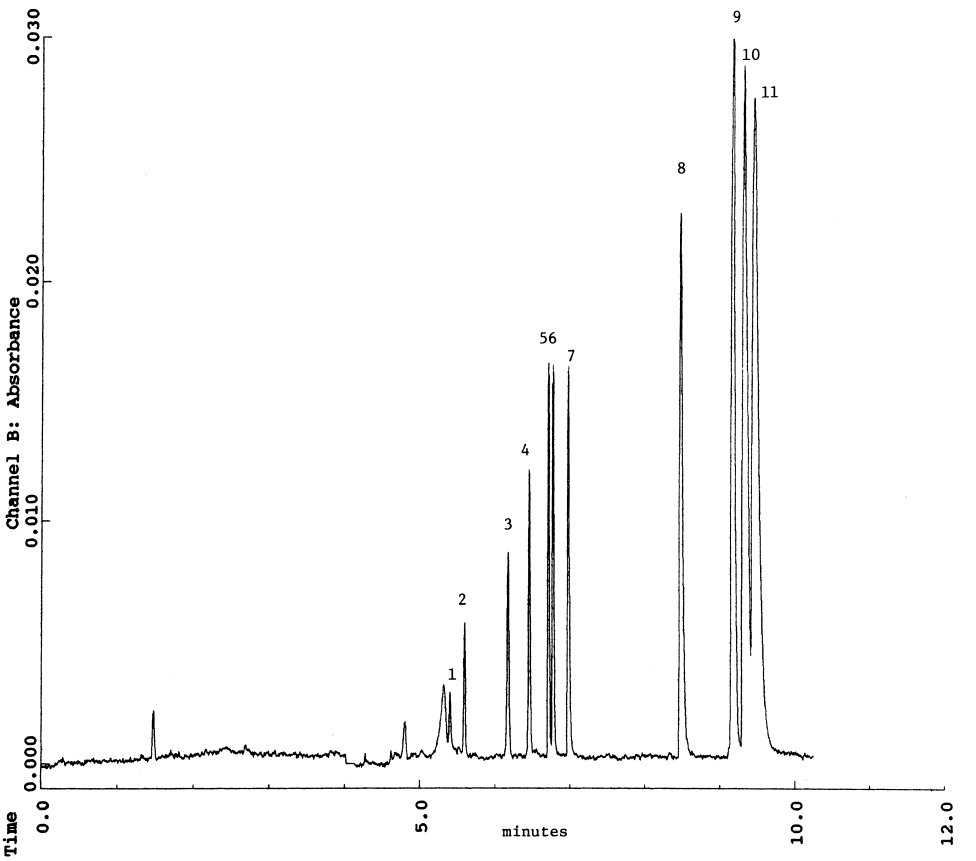

Fig. 1. Separation of 11 DNA fragments in the untreated capillary by electrokinetic injection for 90 s at 5 kV. (Fragments: 1, 72; 2, 118; 3, 194; 4, 234; 5, 271; 6, 281; 7, 301; 8, 603; 9, 872; 10, 1078; 11, 1353 bp).

sodium salt 50 m*M*, boric acid 65 m*M*, HPMC (5 g) and ethidium bromide (1000 μg). These compounds were stirred with the aid of a magnetic stirrer until dissolved completely.

2.3. Chemicals

DNA standard: ds FX174 *Hae*III digest containing 11 fragments at final concentration of 100 μg/ml was obtained from Beckman Instruments. Hydroxypropylmethylcellulose (Viscosity of 2% solution= 3500–5600 cP) and HEPES sodium salt were obtained from Sigma, St. Louis, MO, USA.

2.4. Capillaries

The untreated capillary was obtained from Polymicro Technologies (Phoenix, AZ, USA) and a coated capillary μSil-FC was obtained from J&W Scientific (Folsom, CA, USA).

3. Results and discussion

In DNA work by CE, both the electrokinetic and the hydrodynamic injections are used to deliver the sample into the capillary. In general, the electrokinetic injection gives better stacking provided the sample is free of salts. In practice, PCR products usually contain salts. Fig. 1 shows the separation obtained, by the electrokinetic injection, on the untreated capillary. All of the 11 fragments are well separated including the two closely related fragments 271 and 281 (peaks 5 and 6). This separation is facilitated by the addition of ethidium bromide, which stiffens the DNA structure. However, with

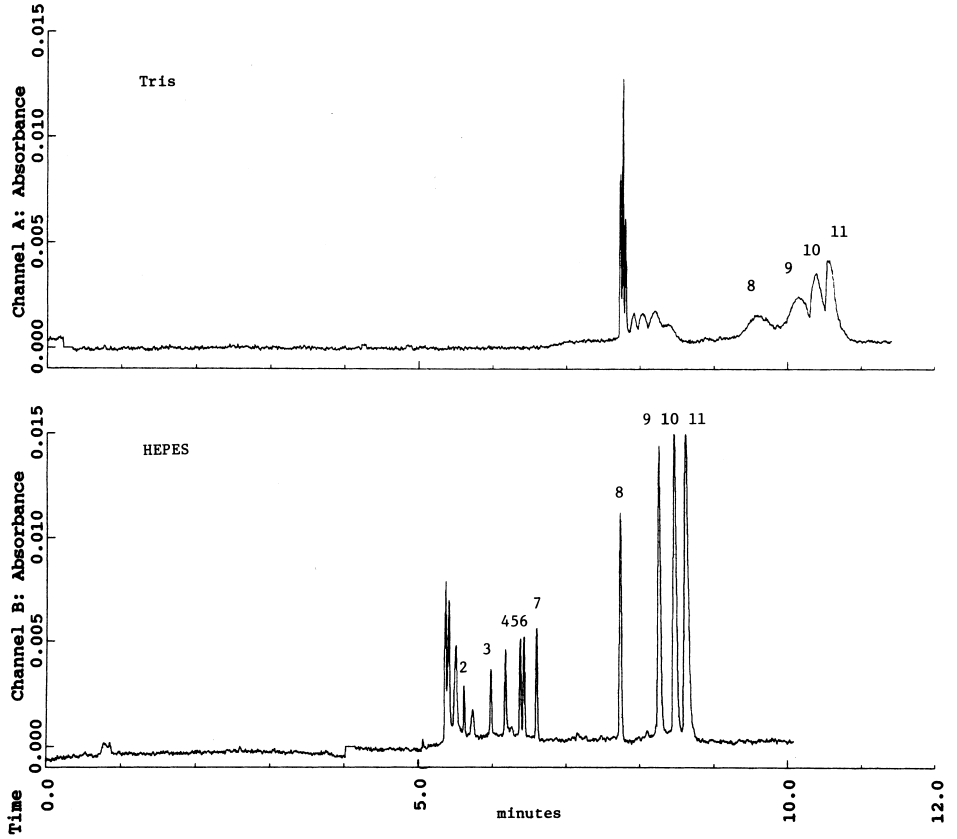

Fig. 2. Effect of buffer type 89 m*M* Tris–89 m*M* borate vs. 50 m*M* HEPES–65 m*M* borate using pressure injection filling 25% of the capillary volume with sample: (top) Tris buffer, and (bottom) HEPES buffer. (Numbers as in Fig. 1).

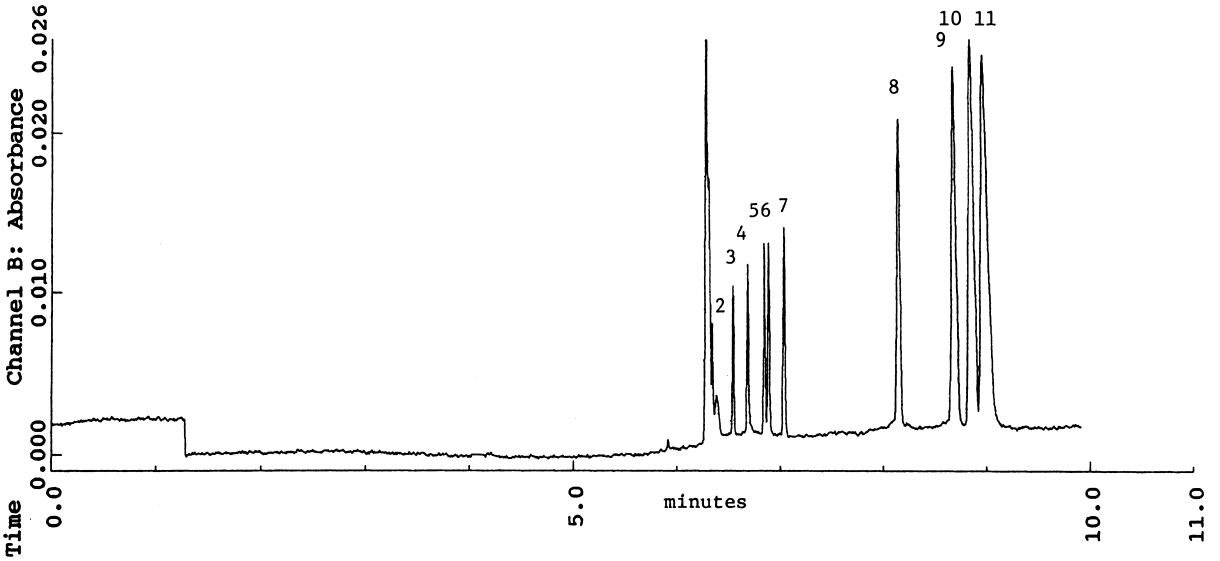

Fig. 3. Sample stacking in the untreated capillary, sample loading at 50% of the capillary volume. (Numbers as in Fig. 1).

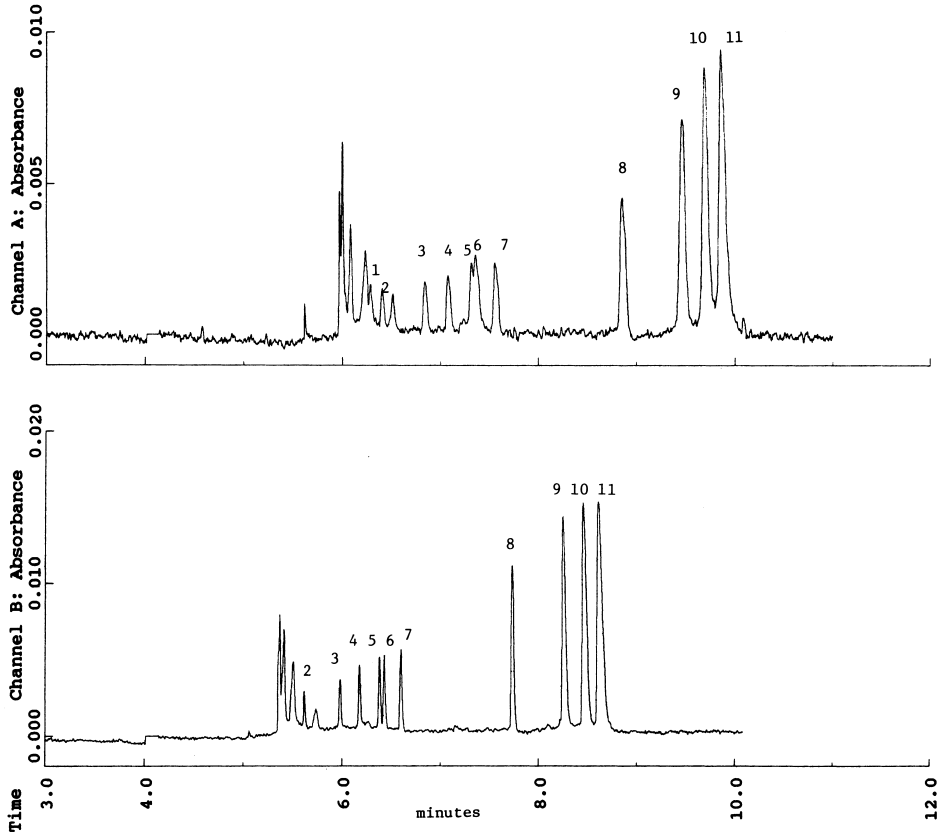

Fig. 4. Comparison of the separation in: (bottom) an untreated capillary (25 cm×75 μm); and (top) the μSil capillary (33 cm×75 μm) at ample loading of 25% of the capillary. (Numbers as in Fig. 1).

repeated injections, the peaks decrease in size presumably due to the electromigration of some counterions from the buffer to the sample cup. On the other hand, this problem is avoided in the hydrodynamic injection with a better ratio of the different peaks. Also, in this type of injection, it is easier to calculate the exact volume of sample being loaded on the capillary. Thus, pressure injection was used in the subsequent experiments.

Tris has been used commonly as a buffer for DNA separation in both CE [1–4,6,7] and gel electrophoresis too. However, in CE a coated capillary is necessary to decrease the EOF and to decrease the capillary wall interaction. Fig. 2 compares the separation of the phage DNA by the Tris buffer to that of the HEPES buffer in an untreated capillary using pressure injection with a large sample loading (25% of the capillary volume). The separation in the Tris

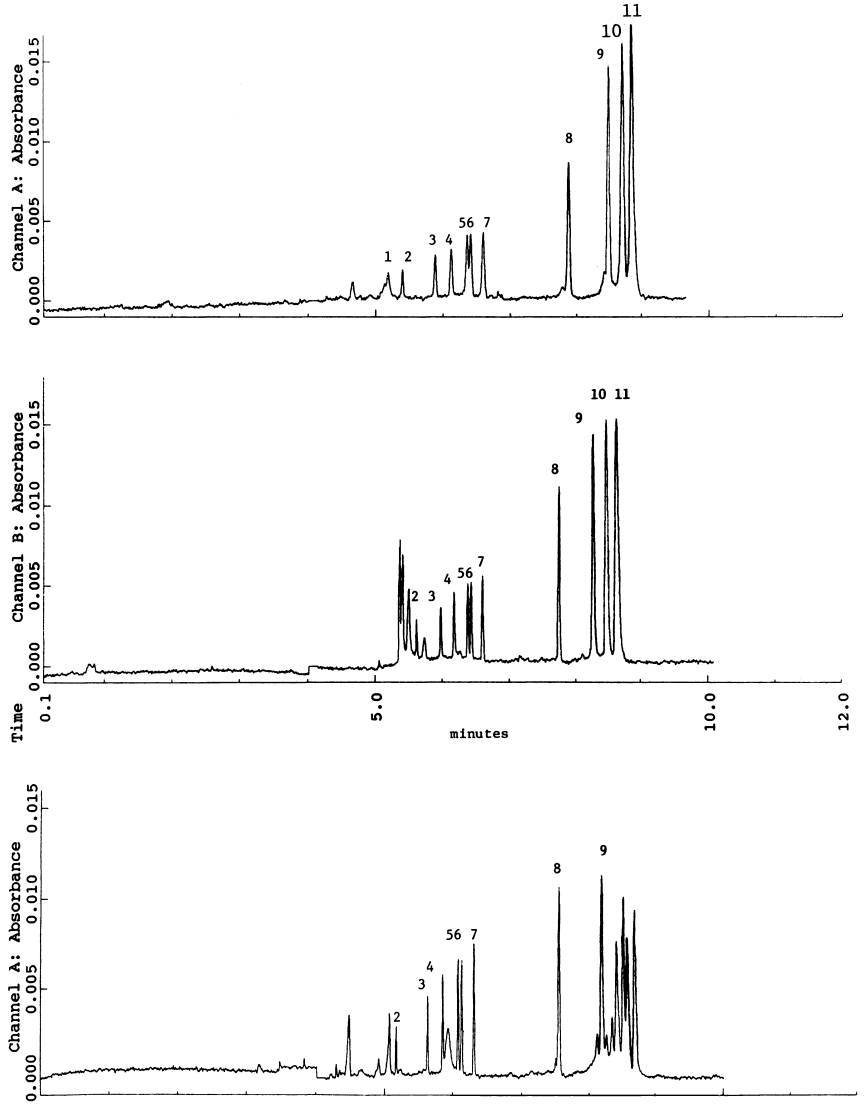

Fig. 5. Effect of sample diluent on the separation: (top) water, (middle) KH_2PO_4 5 mg/1 ml (final concentration), and (bottom) 0.25% NaCl, in untreated capillary with sample loading of 25%. (Numbers as in Fig. 1).

buffer was poor and the peaks were very broad probably due to the interaction of the DNA molecules with the capillary wall. Furthermore, the DNA fragments migrated very slowly in the Tris buffer indicating that they were pushed backwards to the cathode by the EOF. These effects were eliminated by the use of the zwitterionic HEPES buffer. Zwitterionic buffers are known to decrease the capillary wall interaction [9–12] and also help in sample stacking [11,12]. Decreasing the HEPES–borate buffer concentration five times deteriorated the separation. Increasing the HEPES–borate buffer two-fold increased the migration time and increased slightly the peak width, too. Thus, the optimum concentration for the HEPES was about 50 m*M*. Under these conditions, about 50% of the capillary volume can be loaded with sample with good separation, Fig. 3. At this sample loading the capillary effective length decreases to about 8.7 cm. Short capillaries have been shown to remain effective for the separation of DNA [13]. They have the advantage of speeding up the analysis.

The comparison of the separation in the untreated to a commercially coated capillary is illustrated in Fig. 4. The separation, in general, is similar although the analysis was optimized for the untreated capillary. In addition to that the cost of the untreated capillary is negligible compared to the commercially coated one.

Sample concentration on the capillary (stacking) is very important for DNA detection at 254 nm relative to that by the laser induced fluorescence. Fig. 5 shows a slightly improved stacking by addition of KH_2PO_4 to the sample compared to simple dilution with water, especially for the fragments 5 and 6. The addition of KH_2PO_4 also stabilizes the sample so multiple repeat injections (by pressure) can be used from the same cup. Addition of small amounts of NaCl (0.25%) to the sample also improved the separation of the small size fragments up to 700 base pairs (bp). In general, the pressure injection gives comparable separation similar to that of the electrokinetic one. Unfortunately, the potassium phosphate and the sodium chloride are not compatible with the electrokinetic injections. In addition to that, KH_2PO_4 introduces a few early peaks, which can interfere in the UV-detection with the 72 fragment especially at high sample loading, Figs. 2 and 3. On the other hand, these peaks can serve as internal standards. Poor resolution due to multiple injections or prolonged standing on the instrument can be improved or restored by the addition of potassium phosphate. The KH_2PO_4 and the salts may help solubilize or stabilize the DNA structure through the hydrogen bonding (the binding of the two strands) rather than help in true stacking.

The data presented here show that HEPES buffer allows the use of untreated capillaries for DNA separation using either hydrodynamic or electromigration injection. Furthermore, the addition of potassium phosphate can improve the stacking for the hydrodynamic injection. A similar data was obtained by using poly(ethyleneoxide) molecular weight 2 000 000 as a polymer in place of the HPMC.

References

[1] Y. Baba, N. Ishimaru, K. Samata, M. Tsuhako, J. Chromatogr. A 653 (1993) 329.
[2] H.E. Schwartz, K. Ulefldер, F. Sunzeri, M.P. Busch, R.G. Brownlee, J. Chromatogr. 559 (1991) 267.
[3] H.E. Schwartz, K.J. Ulefldер, Anal. Chem. 64 (1992) 1737.
[4] Y. Kim, M.D. Morris, Anal. Chem. 66 (1994) 1168.
[5] A.E. Barron, W.M. Sunada, H.W. Blanch, Electrophoresis 16 (1995) 64.
[6] Q. Gao, E.S. Yeung, Anal. Chem. 70 (1998) 1382.
[7] E.N. Fang, E.S. Yeung, Anal. Chem. 67 (1995) 1913.
[8] W.M. Sunada, H.W. Blanch, Electrophoresis 18 (1997) 2243.
[9] M.M. Bushey, J.W. Jorgenson, J. Chromatogr. 480 (1989) 301.
[10] G. Mandrup, J. Chromatogr. 604 (1992) 267.
[11] Z.K. Shihabi, M. Friedberg, J. Chromatogr. A 807 (1998) 129.
[12] Z.K. Shihabi, J. Chromatogr. A 817 (1998) 25.
[13] K.C. Chan, G.M. Muschik, H.J. Issaq, J. Chromatogr. B 695 (1997) 113.