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Rapid high-performance liquid chromatography of nucleic acids with polystyrene-based micropellicular anion exchangers

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SUMMARY

Nucleic acids were separated by ion-exchange chromatography on 30 × 4.6 and 100 × 4.6 mm columns packed with a micropellicular anion exchanger made of 3-μm rigid polystyrene-based non-porous microspheres with a covalently bound hydrophilic layer and DEAE functional groups at the surface. The stationary phase particles showed negligible swelling in methanol according to permeability measurements with water and methanol. Nucleic acids and their fragments including synthetic single-stranded oligonucleotides, linear, nicked and supercoiled DNAs as well as DNA restriction fragments were separated in less than 5 min, a time scale that is much smaller than that of conventional high-performance liquid chromatographic analysis for such samples. When only buffer and sodium chloride were used in the eluent for the separation of double-stranded DNA restriction fragments pGEM-3Z/Taq I, electrophoretic analysis of the effluent revealed the presence of smaller fragments in the bands of the larger ones. Upon addition of ethylenediaminetetraacetic (EDTA) salt to the eluent, however, such contamination by shorter fragments was no longer observed. In the absence of EDTA, magnesium chloride in the eluent at a concentration of 1 m*M* precluded the separation of the restriction fragments under otherwise identical chromatographic conditions.

INTRODUCTION

Rapid developments in recombinant DNA technology have created the need for isolating and purifying nucleic acids. The standard methods to purify plasmid DNA, DNA restriction fragments, and oligonucleotides are time consuming and labor intensive. In the case of plasmid DNA, a cesium chloride gradient containing ethidium bromide is frequently used. Although the supercoiled DNA can be separat-

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ed from other types of DNA, it is often difficult to purify it from RNA[1]. Excluding the time and labor in processing the sample before and after centrifugation, it takes at least 3 h to complete the centrifugation[1]. Restriction fragments are typically separated by gel electrophoresis, which has excellent resolution but low loading capacity. Preparative separation of DNA with high resolution requires extended time and recovery is often problematic. Certain unidentified substances from the gel may accompany the purified nucleic acid fractions and inhibit some enzymatic reactions; therefore, the DNA recovered after gel electrophoresis has often to be further purified.

Much of the importance of synthetic oligonucleotides derives from their increasingly widespread use in different areas, such as the site-specific mutagenesis[2–5], sequence-specific hybridization[6], priming of different enzymatic reactions[7,8] and affinity chromatography[9]. With an automated DNA synthesizer, oligonucleotides containing up to 60 bases can be made easily. The purity of a 60-mer thus obtained, however, is only 54–60% and its purification is carried out by chromatography or gel electrophoresis. Electrophoretic separation methods employing capillary tubes[10,11] are expected to bring about improvements, yet the loading capacity of these techniques are believed to be very low. For those reasons there is a need to develop appropriate high-performance liquid chromatographic (HPLC) methods, to complement both slab and capillary electrophoresis.

Recent advances in HPLC, particularly the introduction of novel microparticulate sorbents, have already extended the scope of its applications in nucleic acids research. However, HPLC of DNA fragments has been hampered by low diffusivities of the biopolymer molecules and their entrapment in the cavernous interior of conventional sorbents with the result of poor separation efficiency and low recovery. Further developments are expected from the introduction of micropellicular sorbents which are made of spherical, fluid-impervious support particles of a few microns in diameter with an appropriate retentive coating at the surface. The lack of internal pore structure offers advantages such as absence of significant intraparticulate diffusion resistances and good recovery[12,13] and these sorbents have been successfully used for rapid separation of proteins and peptides in different modes of chromatography[14–18].

This report examines the potential of a polymer-based micropellicular ion-exchange sorbent in rapid analysis as well as micropreparative purification of nucleic acids. Micropellicular anion-exchanger with DEAE ligates was prepared with a spherical, highly cross-linked polyaromatic support of 3.3 μm particle diameter[18]. Thus the operating pH range of the column could be extended to alkaline eluents to reduce inter- and intra-molecular interactions, which can impede DNA separations[19–21]. Furthermore, the use of the polymeric support also allows cleaning of the column with sodium hydroxide solution.

EXPERIMENTAL

Chemicals

Reagent-grade fuming nitric acid, granulated tin metal, sodium hydroxide and sodium nitrite were purchased from Mallinckrodt (Paris, KY, U.S.A.). Acrylamide and bisacrylamide, both electrophoresis grade, ammonium persulfate and

N,N,N',N'-tetramethylethylenediamine were from Bio-Rad (Richmond, CA, U.S.A.). Triglycidoxyglycerol was purchased from Polysciences (Warrington, PA, U.S.A.). Trizma base [Tris(hydroxymethyl)aminomethane], ethidium bromide, and β-lactoglobulin B were purchased from Sigma (St. Louis, MO, U.S.A.). Sodium chloride, sulfuric acid, hydrochloric acid, EDTA, boric acid and N,N-dimethylformamide were obtained from Fisher Scientific (Springfield, NJ, U.S.A.). Diethylethanolamine was from Aldrich (Milwaukee, WI, U.S.A.) and the other solvents and salts were purchased from J.T. Baker (Phillipsburg, NJ, U.S.A.).

Nucleic acids and enzymes

pGEM-3Z plasmid DNA was purchased from Promega (Madison, WI, U.S.A.) and pXbs201 plasmid DNA was a gift by Dr. D. D. Brown of Carnegie Institution of Washington (Baltimore, MD, U.S.A.). The plasmids were amplified in bacterial strain DH1 and purified by ultracentrifugation with cesium chloride gradient[22]. Oligonucleotides, except the mixture of p(dT)12–18, which was purchased from P-L Biochemicals (Milwaukee, WI, U.S.A.), were synthesized by the phosphoramidite method on a Model 380B DNA synthesizer (Applied Biosystems, Foster City, CA, U.S.A.) with reagents supplied by the manufacturer. The sequences of the SQ1 and H3H-22 oligonucleotides were 5'-GTACTAGTGTCATCTAGTGTCA-3' and 5'-GCATCGCGTGTGCTTTCCGCCG-3', respectively. The single-stranded DNA used in the study, which is a 12-mer, was also synthesized by the same synthesizer and the double stranded DNA was obtained by mixing equal molar amounts of the pairing 12-mers at 37°C for 5 min and then cooled down to room temperature. Restriction endonucleases, BamHI, HindIII, MspI, and TaqI, were purchased from Promega and used according to the manufacturer's instructions. Cleaving of pGEM-3Z with MspI yielded eleven fragments shown in Fig. 8A. Upon cleaving pGEM-3Z with TaqI, five fragments were obtained but only the three major fragments containing 469, 763 and 1447 base-pairs were seen on electropherogram and chromatogram (*cf.* Figs. 6A–D). All enzymes were removed by phenol extraction after cleaving.

Stationary phase

Highly cross-linked polyaromatic microspheres having a mean particle diameter of 3.3 μm, as measured by electronmicroscopy, were prepared by suspension polymerization of styrene and divinylbenzene[18]. The particles were superficially nitrated, reduced and then reacted with diethylethanolamine in the presence of glycidoxyglycerol as described belows.

Nitration. A 10-g amount of dry polystyrene particles was dispersed in 50 ml of N,N-dimethylformamide by sonication. A 60-ml volume of fuming nitric acid and 20 ml of concentrated sulfuric acid were placed into a 300-ml three-neck flask equipped with a stirrer and a thermometer and cooled by an ice bath. The suspension was added slowly to the contents of the flask under stirring at 5–10°C. Thereafter the reaction mixture was stirred at that temperature for 3 h and subsequently heated at 60°C for 3 h. The nitrated product was filtered on a sintered-glass filter, washed with water, 0.1 *M* sodium hydroxide, water and dimethylformamide.

Reduction. Air-dried particles from the above reaction were dispersed in 60 ml of dimethylformamide by using the above reaction flask and cooled in an ice bath,

and 4 g of granulated tin metal were added. From a dripping funnel 100 ml of concentrated hydrochloric acid were added over 1 h. The reaction mixture was stirred at room temperature for another hour, and subsequently heated at 90°C for 9 h. At the end, the product was filtered, and washed with concentrated sodium hydroxide, water and methanol. The particles were dried at 60°C for 2 h.

Diazotization and hydrolysis. A 360-ml volume of 16% (v/v) aqueous sulfuric acid was placed in a 1-l three-neck flask equipped with a stirrer and a thermometer. Then, 100 ml of an aqueous suspension containing 10 g of the product from the previous reaction step were added to the flask under stirring. Subsequently 400 ml of cold water were added to the well-mixed mixture and the reaction flask was immersed in an ice bath. After stirring for an additional 15 min, a cold solution of 70 ml water and 36 g of sodium nitrite was added very slowly in about 1 h. The stirring was continued for another 20 min in the ice bath and followed by stirring at 45°C for 2 h. The product was filtered and washed in a sintered-glass filter with water and dimethylformamide. The particles were dried in the oven at 80°C for 5 h.

Reaction with diethylethanolamine and triglycidoxyglycerol. The product of previous step was dispersed in a mixture of 80 ml dimethylformamide, 50 ml diethylethanolamine and 20 ml triglycidoxyglycerol. The mixture was heated at 90°C for 18 h with stirring. The suspension was filtered and washed with water and acetone. The particles were dried in the oven at 60°C for 3 h.

The reproducibility of the surface treatment was measured by the chromatography of DNA fragments under conditions given in Fig. 8. Three out of five batches of the stationary phase yielded essentially identical chromatograms. The permeability of the columns with 20 m*M* Tris buffer as the mobile phase varied less than 5%.

Columns. In most instances 30 × 4.6 mm I.D. column made of 1/4 in. 316 stainless-steel tubing with 2-μm stainless-steel end frits were used. Some experiments were carried out with a 100 mm long, otherwise identical column. All the columns were slurry-packed at 850 bar by using an air-driven fluid pump Model DSHT-300 from Haskel (Burbank, CA, U.S.A.). Methanol was used for the slurry and also as the packing fluid.

Chromatograph

HPLC was performed on a Hewlett-Packard (Palo Alto, CA, U.S.A.) 1090 liquid chromatography system equipped with an oven, an auto injector and a diode-array detector. The chromatograms were stored and evaluated by Hewlett-Packard Model 9000-300 and 9153B data system.

Electrophoresis

The HPLC fractions, usually between 0.5 and *ca.* 1 ml, were collected manually following the detector signal and adjusted to have 0.5 *M* sodium chloride. After addition of two volumes of 95% aqueous ethanol the DNA precipitated and was recovered by centrifugation. The DNA pellet was dried and resuspended in 10 μl of loading solution containing 5% (v/v) glycerol, 10 m*M* EDTA (pH 8.0), 0.1% (w/v) sodium dodecyl sulfate and 0.01% (w/v) bromophenol blue. The DNA solution was subjected to electrophoresis at 10 V/cm for 5 h by using a 20 cm long and 1.0 mm thick 8% polyacrylamide gel slab made from acrylamide and bisacrylamide at a ratio of 37.5:1, 0.1% (w/v) ammonium persulfate and 0.05% (v/v) N,N,N′,N′-tetramethyl-

ethylenediamine, a TBE buffer containing 89 m*M* Tris-borate, 89 m*M* boric acid and 2 m*M* EDTA. After electrophoresis, the gel was stained with 0.5 mg/ml of aqueous ethidium bromide solution for 10 min and photographed on an UV transilluminator (UVP, San Gabriel, CA, U.S.A.).

RESULTS AND DISCUSSION

Characterization of the column

The permeability of the 30 × 4.6 mm I.D. column was investigated by measuring the pressure drop at various flow-rates of water and methanol as described previously[18]. It is seen from the results depicted in Fig. 1 that the pressure drop is a linear function of the flow-rate at least at inlet pressures up to 300 bar. From the Kozeny–Carman[18] equation with a particle diameter of 3.3 μm, the interstitial porosity[23] of the column was calculated as 0.375 and 0.342 in water and methanol, respectively. The decrease of specific permeability in contact with methanol corresponded to only a 1.7% increase in particle diameter with respect to that in water. Perfusion of the column alternatively with methanol and water did not have any untoward effect on the column efficiency.

In order to estimate the loading capacity of the 30 × 4.6 mm I.D. micropellicular DEAE column for biopolymers, the adsorption isotherm of β-lactoglobulin B was measured by frontal chromatography as described in the literature[24,25]. The experiments were carried out at room temperature with protein solutions in 20 m*M* Tris · HCl buffer, pH 8.0, at a flow-rate of 0.15 ml/min. The saturation value of β-lactoglobulin B was found to be about 9 mg per ml of stationary phase volume and this compares to protein binding capacity of 40 and 20 mg/ml for conventional porous particles having 250 and 550 Å mean pore diameter, respectively[26]. Therefore such micropellicular columns may also be used for preparative purification of proteins and nucleic acids.

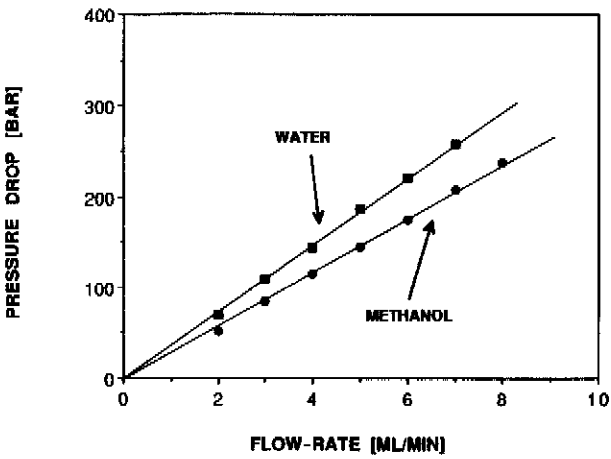


Fig. 1. Plots of pressure drop *versus* flow-rate of water and methanol at 25°C. Column, 30 × 4.6 mm I.D. packed with 3.3-μm micropellicular anion exchanger.

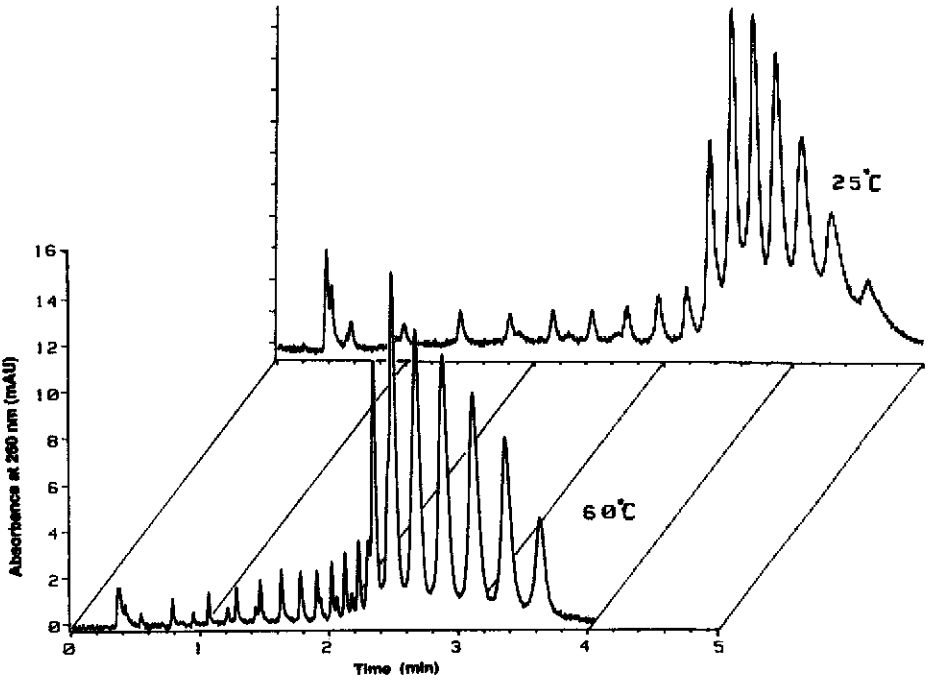


Fig. 2. Chromatograms of the crude products of p(dT)12–18 synthesis at column temperature of 25 and 60°C. Column, 100 × 4.6 mm I.D. packed with 3.3-μm micropellicular DEAE sorbent; flow-rate, 2.0 ml/min; buffer A, 20 m*M* Tris · HCl, pH 9.0; buffer B, 1 *M* NaCl in buffer A; linear gradient from 5 to 30% buffer B in 1.5 min followed by 30 to 45% buffer B in 4.5 min; sample size, 1 μg.

Synthetic oligonucleotides

Fig. 2 shows separations of a mixture of p(dT) oligonucleotides which contains 12 to 18 bases according to the supplier. The short separation time of less than 5 min illustrates that columns packed with micropellicular sorbents offer a means to reduce the time of such analysis to minutes from hours needed when using conventional columns[19,27]. As shown, the speed and efficiency of the separation can be further increased by operating at 60°C. Comparison of the two chromatograms confirms the predictions regarding the benefits of elevated temperatures in analytical HPLC of biopolymers that have recently been discussed in the literature[28].

Solid-phase synthesis of oligonucleotides is widely carried out with automated DNA synthesizers. The product must be purified preferably by HPLC in order to remove various oligonucleotide side products which contaminate the main product. Columns packed with micropellicular sorbents are very promising to bring about such purification rapidly. Fig. 3 shows the chromatogram of an oligonucleotide mixture in which the main product is a 48-mer from an automated DNA synthesizer.

Despite the high resolving power of gel electrophoresis, it is difficult, if not impossible to resolve by this technique DNA fragments that have the same chain length even if they differ in base composition. On the other hand, such separations can be readily performed by the use of the HPLC technique described here as il-

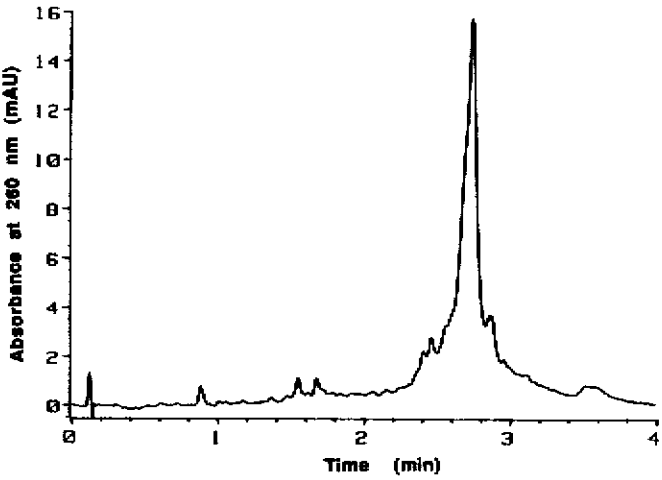
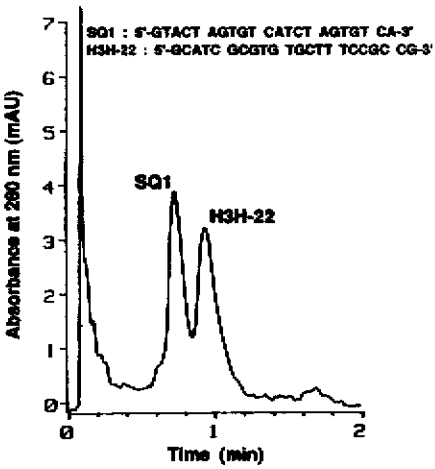


Fig. 3. Chromatogram of a crude 48-mer synthetic oligonucleotide. Column, 30 × 4.6 mm I.D.; flow-rate, 2.0 ml/min; buffer A, 20 m*M* Tris·HCl, pH 8.0; buffer B, 1 *M* NaCl in buffer A; linear gradient from 10 to 90% buffer B in 5 min; temperature, 60°C; sample size, 1 μg.

lustrated in Fig. 4. The two 22-mer oligonucleotides of different base composition, whose molecular weights are different only by 0.68%, are well resolved and the time of separation is less than 2 min.

Single-stranded DNA is usually separated from double-stranded DNA by gel electrophoresis or by liquid chromatography on a hydroxyapatite column. Fig. 5 shows a baseline separation of such a mixture on the micropellicular DEAE stationary phase in less than 4 min.



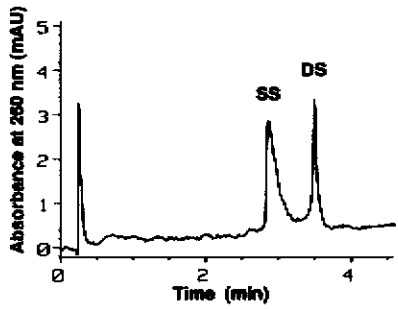


Fig. 4. Separation of two 22-mer oligonucleotides of different base composition. Chromatographic conditions as in Fig. 3, except linear gradient from 3 to 38% buffer B in 3 min; sample size, 0.5 μg.

Fig. 5. Separation of 12-mer single-stranded (SS) and double-stranded (DS) DNA. Column, 30 x 4.6 mm I.D.; flow-rate, 2.0 ml/min; buffer A, 1 m*M* EDTA in 20 m*M* Tris · HCl, pH 8.0; buffer B, 1 *M* NaCl in A; linear gradient from 10 to 15% buffer B in 5 min; temperature, 25°C; sample 0.2 μg of each component.

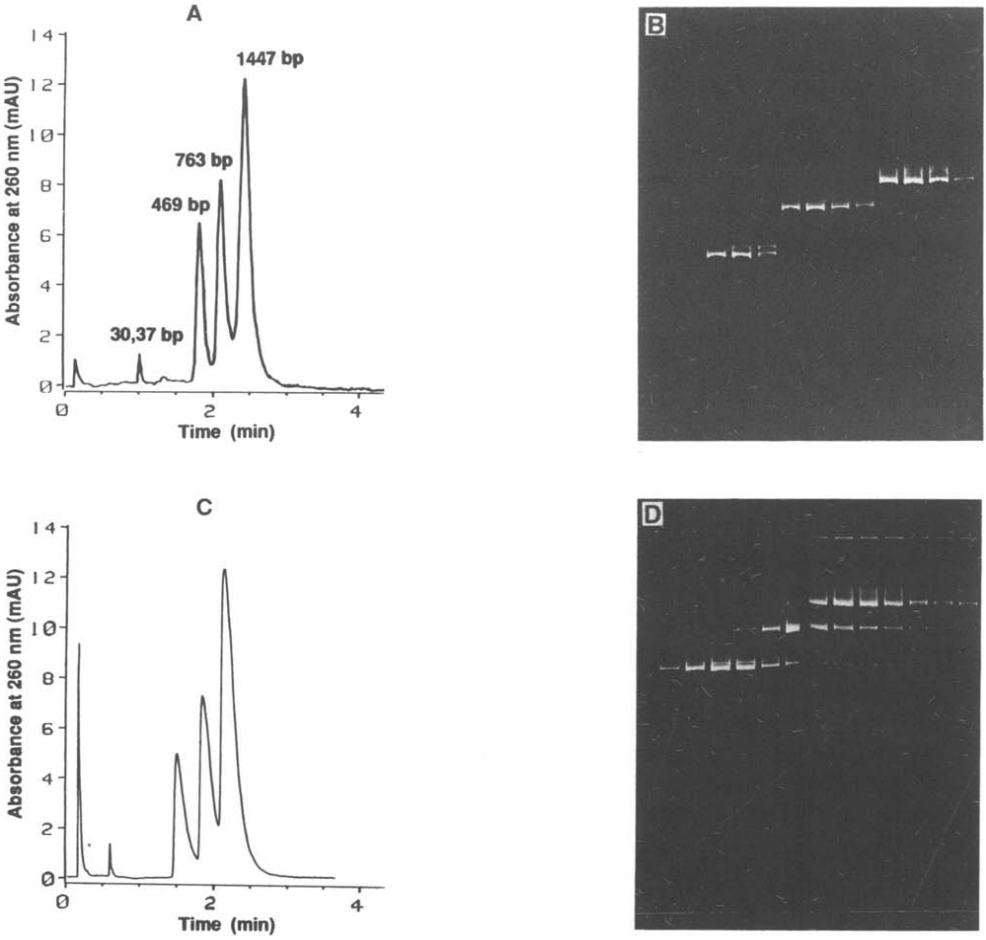


Fig. 6. Separation of DNA fragments from the digest of pGEM-3Z DNA by TaqI endonuclease. (A) Chromatogram was obtained under conditions as in Fig. 5 except linear gradient from 30 to 45% buffer B in 0.5 min followed by 45 to 48% buffer B in 4.5 min was used; sample size, 2 μg; bp = base pairs. (B) Electropherograms of the fractions collected from (A). (C) Chromatogram obtained under conditions as in (A) except no EDTA was added. (D) Electropherograms of the fractions collected from (C).

Restriction fragments

Enzymatic digestion of DNA with a restriction endonuclease yields a mixture of well-defined DNA fragments. The purification of specific restriction fragments is necessary for subcloning, DNA sequencing, and sequence-specific hybridization as well as in many other studies. The separation of such mixture on the micropellicular DEAE column is illustrated in Fig. 6A. It is seen that fragments containing less than 1500 base pairs can be readily separated in less than 5 min. In contradistinction, similar separations may require hours with conventional ion-exchange chromatography on porous DEAE stationary phase[29] or by reversed-phase ion-pair chromatography[30].

The chromatogram of the pGEM-3Z/TaqI restriction fragments in Fig. 6C shows a reasonably good separation of the three major DNA fragments when Tris buffer and NaCl were used in the eluents. However, analysis of the effluent fractions by polyacrylamide gel electrophoresis in Fig. 6D shows that the peaks of the larger fragments are contaminated by the lower-molecular-weight fragments. A similar phenomenon was observed in other chromatographic systems already and referred to as "cross-contamination"[30–32]. We could not eliminate or reduce this "carry-over" of shorter fragments by the larges ones upon varying the flow-rate, column temperature and/or the eluent pH in the range from 6.0 to 9.0. On the other hand, with 1 m*M* of EDTA in the mobile phase we obtained peaks that were electrophoretically pure as shown in Fig. 6B.

Addition of EDTA to the eluent in DNA chromatography is occasionally recommended in the literature to protect the DNA from digestion by non-specific nucleases[33]. It was suggested that "cross-contamination" was also due to the degrading effect of ubiquitous nucleases which need metal for activity. In our case, however, this explanation is highly unlikely. According to electrophoretic analysis shown in Fig. 6B the lower-molecular-weight fragments, present in the bands of the higher-molecular-weight fragments, appeared on the gel only at the same positions as the less retained lower-molecular-weight fragments formed upon the action of the restriction enzyme. Thus, no other fragments were generated in the course of chromatographic procedure.

It has been known that metal ions are released from the wetted steel parts of the HPLC apparatus[34] into the eluent and the adventitious heavy metal ions could facilitate interactions between the various DNA fragments present in the digest. On the other hand EDTA in the eluent may complex the adventitious metal ions and thereby eliminate the observed "carry-over" phenomenon if it is due to binding of the smaller fragments to the larger ones. In order to examine the effect of a divalent metal ion on the chromatographic separation, experiments were carried out with added metal ions

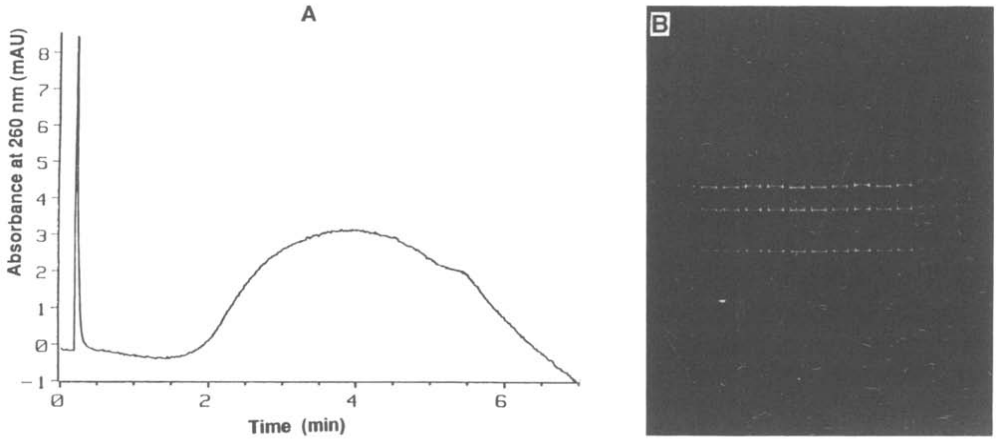


Fig. 7. Chromatogram of pGEM-3Z/TaqI fragments obtained with 1 m*M* $MgCl_2$ in the starting eluent and electropherograms of the fractions. (A) Chromatographic conditions as in Fig. 5 except buffer A, 1 m*M* $MgCl_2$ in 20 m*M* Tris/HCl, pH 8.0; buffer B: 1 *M* NaCl in 20 mM Tris · HCl, pH 8.0; linear gradient from 50 to 100% B in 5 min; sample size, 2 μg. (B) Electrophoretic analysis of the fractions collected from (A).

in the eluent. When 2 m*M* magnesium chloride was present in both the starting eluent and the gradient former the pGEM-3Z/TaqI fragments were not eluted in a gradient from 0 to 1.0 *M* of sodium chloride. When the starting eluent contained 1 m*M* magnesium chloride and no magnesium chloride was added to the gradient former, the DNA fragments eluted in the above gradient as a single broad peak without resolution of the individual fragments as shown in Fig. 7A. Electrophoretic analysis of the fractions collected from the beginning to the end of the peak confirmed, as seen in Fig. 7B, that all three components coeluted. These observations give further support to the hypothesis that the carry-over could be the result of an aggregation of the fragments via metal complexation. In this case the role of the EDTA in the eluent would be to preclude such agglomeration by chelating adventitious metal ions in the chromatographic system. Indeed the chromatographic separation of the restriction fragment mixture pGEM-3Z/MspI obtained by digesting the same plasmid with another restriction enzyme in the presence of 1 m*M* EDTA in the eluent, yielded fractions that did not exhibit carry-over as shown in Fig. 8B. Aggregation of DNA mediated by multivalent ions has been reported previously[35].

"Memory" peaks from the previous injection were reported in the literature[31,32] when some commercial columns were used for the chromatography of DNA fragments. In order to examine the possible occurrence of such phenomenon with the micropellicular column, a blank run was carried out immediately after the analysis of pMEG-3Z/TaqI restriction fragments under conditions given in Fig. 6A. As no "memory" or other ghost peaks appeared on the chromatogram, we conclude that the micropellicular sorbent configuration offers excellent recoveries in such separations in contradistinction to some conventional porous stationary phases. Furthermore, the equilibration time after a gradient run is usually 2 to 5 min on the polystyrene-based micropellicular DEAE column depending on the flow-rate. Thus column regeneration can be performed in a much shorter time than that required for conven-

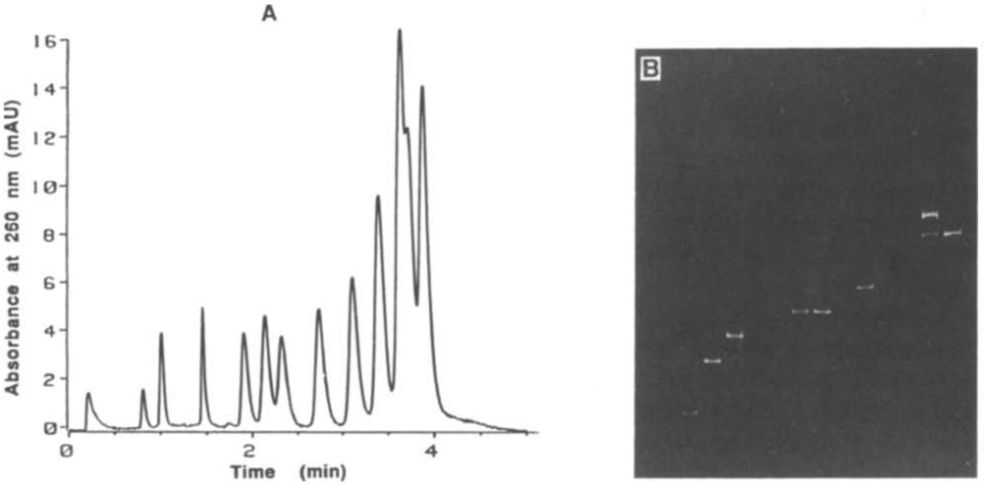


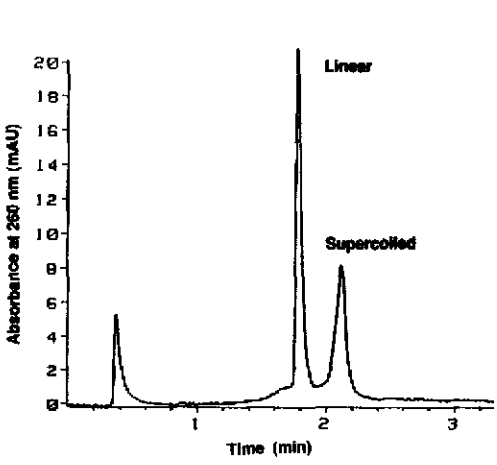
Fig. 8. Separation of DNA fragments from the digest of pGEM-3Z plasmid by MspI endonuclease. (A) Chromatogram obtained under conditions as in Fig. 5 except linear gradient from 33 to 40% buffer B in 1 min followed by 40 to 47% buffer B in 4 min, sample size, 1 μg. (B) Electropherograms of the fractions collected from (A).

tional sorbents according to the literature[31]. This can be readily explained by the rapid mass transfer facilitated by the particular structure of the micropellicular stationary phase in which the thin retentive layer confined to the surface of a fluid impervious support.

About the time when this manuscript was submitted a publication by Kato *et al.*[36] described the use of another kind of micropellicular anion exchanger for the separation of DNA restriction fragments. The authors observed no significant improvement in the resolution upon increasing the temperature from 25 to 65°C. We had similar experience in the chromatography of large double-stranded restriction fragments in contradistinction to the effect of temperature on the separation of the relatively small single-stranded oligonucleotides shown in Fig. 3.

Supercoiled DNA and its conformational isomers

After removal of high-molecular-weight cellular RNA and DNA, plasmid DNA is further purified by separating its supercoiled and nicked forms. In experiments with porous ion-exchange stationary phases, it was noted that size-exclusion effects interfered with the purification of supercoiled plasmid DNAs[37] when the mean pore size of the stationary phase was not larger than 2000 Å. Therefore sorbents having even larger pore dimensions would be needed to eliminate such size-exclusion effects as well as entrapment of some sample components in the cavernous interior of conventional stationary phase particles. Although sorbents having mean pore diameters of 4000 Å are now commercially available, the non-uniformity of the pore size distribution and the relatively poor mechanical stability of the support may limit their use. On the other hand, non-porous sorbents are not plagued with such problems and are, therefore, particularly suitable for the chromatography of megamolecules, *i.e.*, molecules having molecular weights higher than 10^6 daltons. This is illustrated by the



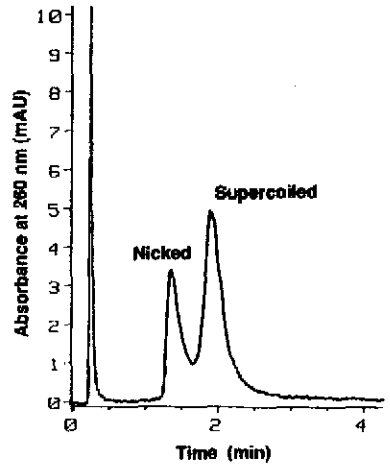


Fig. 9. Separation of linear and supercoiled DNA. Chromatographic conditions as in Fig. 6 except linear gradient from 40 to 55% buffer B was used in 5 min; sample, pGEM-3Z and pGEM-3Z/HINDIII, 0.25 μg each.

Fig. 10. Separation of nicked and supercoiled DNA. Chromatographic conditions as in Fig. 6 except linear gradient from 45 to 50% buffer B in 5 min; sample size, 0.3 μg each.

chromatogram in Fig. 9 which shows a separation of pGEM-3Z from linearized form pGEM-3Z/HINDIII on the micropellicular DEAE column in 3 min. This is a rather rapid separation since such HPLC analysis with conventional columns usually require at least 30 min according to the literature[31,33]. Moreover, by using this approach, the open circular, or nicked form, could also be rapidly separated from the supercoiled form, as depicted by the chromatogram in Fig. 10.

CONCLUSIONS

The results of this study have demonstrated that polystyrene-based micropellicular DEAE ion exchangers are well suited for rapid, high-efficiency separation of nucleic acids and their fragments and require very short postgradient column reequilibration. Their use therefore is associated with a considerable saving in time, eluent consumption and instrument usage, which can be important in routine analysis process monitoring and method development.

The microparticulate sorbent configuration also facilitates high recoveries of the sample components so that "carry-over" and "memory" effects do not interfere with the analysis and the efficiency of micropreparative work is enhanced.

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REFERENCES

1 S. J. Garger, O.M. Griffith and L. K. Grill, *Biochem. Biophys. Res. Commun.*, 117 (1983) 835.
2 W. Kramer and H.-J- Fritz, *Methods Enzymol.*, 154 (1987) 350.
3 T. A. Kunkel, J. D. Roberts and R. A. Zakour, *Methods Enzymol.*, 154 (1987) 367.
4 W. Mandecki, *Proc. Natl. Acad. Sci. U.S.A.*, 83 (1986) 7177.
5 R. M. Myers, L. S. Lerman and T. Maniatis, *Science (Washington, D.C.)*, 229 (1985) 242.
6 A. M. Ginsberg, B. O. King and R. G. Roeder, *Cell*, 39 (1984) 479.
7 K. B. Mullis and F. Faloona, *Methods Enzymol.*, 155 (1987) 335.
8 L. B. Agellon and T. T. Chen, *Gene Anal. Techn.*, 3 (1986) 86.
9 J. T. Kadonaga and R. Tjian, *Proc. Natl. Acad. Sci. U.S.A.*, 83 (1986) 5889.
10 A. S. Cohen, S. Terabe, J. A. Smith and B. L. Karger, *Anal. Chem.*, 59 (1987) 1021.
11 A. S. Cohen, D. Najarian, J. A. Smith and B. L. Karger, *J. Chromatogr.*, 458 (1988) 323.
12 Y. Kato, K. Nakamura and T. Hashimoto, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 8 (1985) 154.
13 D. J. Burke, J. K. Duncan, C. Siebert and G. S. Ott, *J. Chromatogr.*, 359 (1986) 533.
14 K. Kalghati and Cs. Horváth, *J. Chromatogr.*, 398 (1987) 335.
15 L. Varády, K. Kalghatgi and Cs. Horváth, *J. Chromatogr.*, 458 (1988) 207.
16 Y. Kato, J. Kitamura, A. Mitsui and T. Hashimoto, *J. Chromatogr.*, 398 (1987) 327.
17 R. Janzen, K. K. Unger, H. Giesche, J. N. Kinkel and M. T. W. Hearn, *J. Chromatogr.*, 397 (1987) 91.
18 Y.-F. Maa and Cs. Horváth, *J. Chromatogr.*, 445 (1988) 71.
19 H. Rockstroh, W. Wangler and Th. Ackermann, *J. Chromatogr.*, 442 (1988) 401.
20 J. A. Thompson, *BioChromatography*, 1 (1986) 16.
21 Y. Kato, T. Kitamura, A. Mitsui, Y. Yamasaki, T. Hashimoto, T. Murotsu, S. Fukushige and K. Matsubara, *J. Chromatogr.*, 447 (1988) 212.
22 T. Maniatis, E. F. Fritsch and J. Sambrook (Editors), *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring, NY, 1982.

23 Cs. Horváth and H.-J. Lin, *J. Chromatogr.*, 126 (1976) 401.
24 J.-X. Huang and Cs. Horváth, *J. Chromatogr.*, 406 (1987) 275.
25 J.-X. Huang and Cs. Horváth, *J. Chromatogr.*, 406 (1987) 285.
26 S. Chang, R. Noel and F. Regnier, *Anal. Chem.*, 48 (1976) 1839.
27 D. Scanlon, J. Haralambidis, C. Southwell, T. Turton and C. Tregear, *J. Chromatogr.*336 (1984) 189.
28 F. Antia and Cs. Horváth, *J. Chromatogr.*, 435 (1988) 1.
29 Y. Kato, M. Sasaki and T. Hashimoto, *J. Chromatogr.*, 265 (1983) 342.
30 S. Eriksson, G. Glad, P. Pernemalm and E. Westman, *J. Chromatogr.*, 359 (1986) 265.
31 D. J. Stowers, J. M. B. Keim, P. S. Paul, Y. S. Lyoo, M. Merion and R. M. Benbow, *J. Chromatogr.*, 444 (1988) 47.
32 M. Merion and W. Warren, *BioTechniques*, 7 (1989) 60.
33 J. A. Thompson, *BioChromatography*, 2 (1987) 4.
34 S. M. Cramer, B. Nathanael and Cs. Horváth, *J. Chromatogr.*, 295 (1984) 405.
35 M. Mandelkern, N. Dattagupta and D. M. Crothers, *Proc. Natl. Acad. Sci. U.S.A.*, 78 (1981) 4294.
36 Y. Kato, Y. Yamasaki, A. Onaka, T. Kitamura and T. Hashimoto, *J. Chromatogr.*, 478 (1989) 264.
37 J. A. Thompson, *BioChromatography*, 1 (1986) 68.