Separation of Mononucleotides by Electrophoresis in a Packed Column

Lei Feng[1] and **Fengsheng Zhao**[2,*]

[1]Instrumental Analysis Center, Shanghai Jiao Tong University, 800 Dong Chuan Road, Shanghai 200240, People's Republic of China, and [2]School of Pharmacy, Key Laboratory of Microbial Metabolism in Ministry of Education, Shanghai Jiao Tong University, 800 Dong Chuan Road, Shanghai 200240, People's Republic of China

Abstract

Electrophoresis in a chromatographic column packed with porous gel has been successfully established to preparatively separate proteins and DNA. This method shows good separative performance. Since separation of small polar nucleotides and oligonucleotides is challenging to conventional reversed phase high-performance liquid chromatography (RP-HPLC), it is of significance to establish preparative electrophoresis for laboratory-scale purification of them. In this paper, milligram of model polar samples, cytidine 5'-monophosphate (CMP) and uridine 5'-monophosphate (UMP), could be completely separated by a column (0.6 cm i.d.) packed with Sepharose 6B at pH 3.6 in 120 V/cm electric field, but never be separated without electric field. The orientation and strength of electric field could affect retention, resolution, and recovery of mononucleotides. With cathode at the column outlet and anode at the column inlet, the electric field force on UMP was strong enough to counteract the buffer flow, retain UMP at the column inlet, and markedly enhance the resolution between UMP and CMP. With this electrical retention mode, the maximum sample capacity was 7.5 mg on a column of 40 × 0.6 cm i.d. (bed volume 11.4 mL), which is much more than that of a RP-HPLC column. This electrokinetic method is a potential system for the separation of small polar compounds from natural extracts.

Introduction

Electrophoresis is a highly successful method for the separation of biological substances with high resolution. Electrophoresis in capillaries or gels is commonly used to separate proteins and nucleic acids, but the former is limited for analytical purpose and the latter meets a difficult procedure of sample recovery. Column chromatography is a powerful method for preparative and analytical separation due to its superior scale-up properties. Electrophoresis in a chromatographic column packed with porous gel has been successfully established to separate biological substances, such as proteins (1–4) and DNA (5,6). Online fractionation of the separated samples could be made, but semi-preparation of small polar compounds was limited, because of the construction of reported preparative electrophoresis apparatus (1–6). In the apparatus, the eluent buffer and electrode buffer were separated, and there were cut-off membranes between the electrode buffers and column in order to prevent electrically produced bubbles from entering column. Therefore, the molecular weights of the separated samples must be higher than the membrane cut-off, otherwise the samples would leak into the electrode buffer and lose.

As polar biological substances, small-molecule nucleotides, such as oligonucleotide consisting of 15–20 nucleotides (7,8), peptide nucleic acid (9), and mononucleotide analogs (7,10) could easily transport into cell membrane and thus are promising to be lead compounds in the field of novel drug discovery. However, separation of highly polar compounds is challenging due to the poor retention in conventional reversed-phase high-performance liquid chromatography (RP-HPLC) (11–13). Anion-exchange chromatography is always used in the separation of mono- and oligonucleotides (14,15). Ionic concentration or pH gradient elution is necessary for high resolution and recovery. Hydrophilic interaction chromatography with a titania column was effective, but the unique media in preparative column would be costly (16). Capillary electrophoresis was also useful (15,17), but the capillary-based method could not provide microgram-scale separation, which is indispensable in the determination and identifications afterwards. Some bases and oligonucleotides had been separated in high electric field applied across a small column of 0.5 mm i.d. packed with nonporous RP material (18,19). Although bigger column than capillary was used to give fast and baseline separation, these systems were also limited to analytical fields and must be run under high pressure to minimize bubble formation, which could interrupt the electric current in such a small column.

Laboratory-scale purification of nucleotides is necessary to the research of nucleotide drugs, gene therapy, gene function and the oligonucleotide-modified beads for hybridization array techniques (20). Therefore a semi-preparative electrophoresis system, using low-pressure equipment and porous chromatographic media, is investigated in this paper on the basis of the electrochromatographic system reported by our group recently (21). Cytidine 5'-monophosphate (CMP) and uridine 5'-monophosphate (UMP) (Figure 1) were chosen as model samples to test this technique for the separation of small polar nucleotides. The influences of medium porosity and electric field application modes on the resolution were discussed.

*Author to whom correspondence should be addressed: email fszhao@sjtu.edu.cn

Experimental

Materials

Sepharose 2B {range of fraction [M_r (molecular weight)]: $1 \times 10^4 - 4 \times 10^7$}, Sepharose 6B [range of fraction (M_r): $1 \times 10^4 - 4 \times 10^6$], Sephadex G-75 [range of fraction (M_r): $3 \times 10^3 - 8 \times 10^4$] and Sephadex G-10 [range of fraction (M_r): $< 7 \times 10^2$] were purchased from Pharmacia Inc. (Uppsala, Sweden). Cytidine 5'-monophosphate (CMP) and uridine 5'-monophosphate (UMP) were of analytical grade from Sigma Chemical Co. (Steinheim, Germany). Other reagents, including sodium acetate, acetic acid, and sodium dihydrogen phosphate, were of analytical grade and methanol was of HPLC grade, which were purchased from local sources.

Equipment

Figure 2 shows the semi-preparative electrophoresis equipment, modified from chromatographic system. It comprised two electrode chambers, column, electric power supply, UV detector, fraction collector, and cooling parts. Two electrode chambers, that is, the inlet chamber at the top of the column (6 cm × 4 cm i.d.) and the outlet "T"-shape chamber at the bottom of the column (2 cm × 0.4 cm i.d. for each branch), were separately connected to the two ends of the jacketed glass column (40 cm × 0.6 cm i.d.) with rubber tubes (0.4 cm i.d.). The vertical branch of the outlet chamber was open to free electrolysis bubbles. One horizontal branch was attached to column exit, and the other was connected to a peristaltic pump (Jin Da Biochemical Analytical Instrument Co., Shanghai, China). The pump was used to transport effluent to the UV detector and then a fraction collector (Jin Da Biochemical Analytical Instrument Co.).

Two platinum electrodes (0.5 mm o.d.), attached to DYY-12C electric power supply (Liu Yi Analytical Instrument Co., Beijing, China), were immersed in the mobile phase buffer in two electrode chambers respectively. A positive electric field was applied with anode at the column outlet, in which the electrophoresis direction of anions was the same as the buffer flow. A negative field orientation was with cathode at the column outlet, in which two directions were opposite.

During the operation course, the solution in upper chamber was circulated with the solution in external reservoir at the rate up to 5-fold faster than the column flow rate. The purpose of the circulation were avoiding pH change during electrolysis and taking bubbles out of this chamber. The eluent flew from the column outlet into the bottom chamber. Then it was transported into the UV detector by the peristaltic pump. Since the effluent had to be examined by online detection and fractionated by collection, the eluent in bottom chamber could not be refreshed with external reservoir for avoiding the loss of online detection and collection.

The cooling parts, including two ice-water baths and a column jacket, were indispensable to keep the column temperature below 30°C.

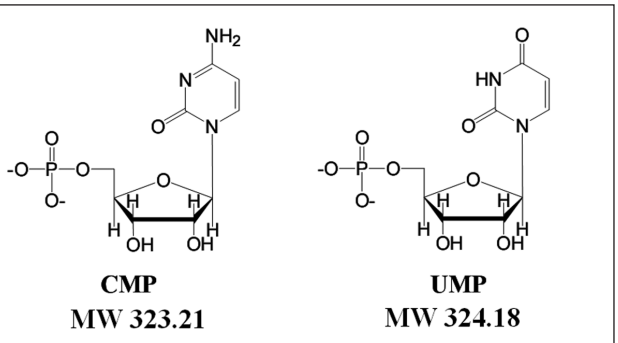

Figure 1. The molecular structure of CMP and UMP.

Experimental course

Sodium acetate and acetic acid (NaOAc-HOAc) buffer of 2 mM was employed as mobile phase solution. Porous gel Sepharose 6B, Sepharose 2B, Sephadex G-10, and Sephadex G-75 were chosen as the media. Columns were packed with media according to common packing procedure. The packed column, two electrode chambers, and UV detector were connected with rubber tubes. Bubbles in the rubber tubes were removed by squeeze and press. CMP (1 mg) and UMP (0.5 mg) in 0.2 mL distilled water were loaded into the well-equilibrated column. The mobile phase solution was added into the column to a fixed height (10 cm) to stabilize the hydrodynamic flow (0.19 mL/min), and the rotary speed of the column outlet pump was adjusted to keep the same pace with the flow rate. The electric power was immediately turned on and the online detection at 254 nm was started. Unless otherwise indicated, the electric field was applied until the experiment was finished. Every 0.8 mL of effluent was collected and analyzed by HPLC.

Analysis

Analysis of fractional collections was performed with Agilent 1100 series HPLC system (Agilent Technologies, Boeblingen, Germany) with a reversed-phase ZORBAX SB C_{18} column (250 × 4.6 mm i.d., 5 µm, Agilent). Methanol and 0.01 M NaH_2PO_4 (5:95, v/v) were used as the mobile phase. Column temperature was maintained at 20°C and flow rate was 0.7 mL/min. The effluent was monitored at 260 nm by diode assay detector (DAD).

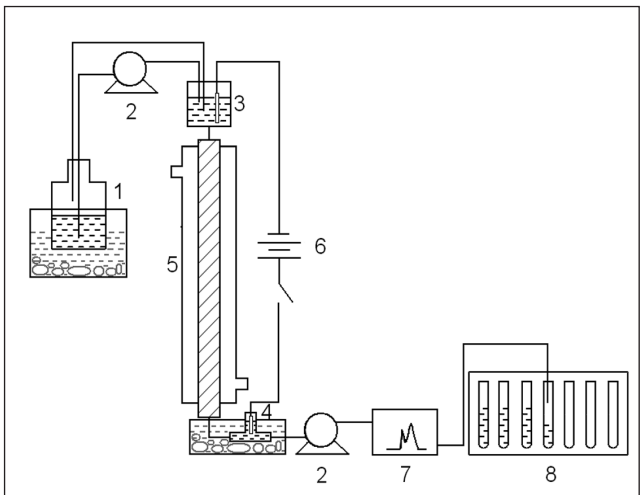

Figure 2. Equipment for electrophoresis. (1) Buffer reservoir with ice bath, (2) peristaltic pump, (3) inlet electrode chamber, (4) outlet electrode chamber with ice bath, (5) jacketed glass column, (6) electric power, (7) detector, and (8) fraction collector.

To evaluate the separation results, the corresponding time of every fraction was plotted against sample concentration analyzed by HPLC. The retention time (R_t) was the time when the corresponding maximum detection signal of the peak appeared. The peak width (W) was denoted by a time period, in which each fraction contained over 2.0 µg/mL CMP or UMP. The resolution (R_S) between plotted peaks was calculated as following:

$$R_S = 2(R_{t2} - R_{t1}) / (W_1 + W_2)$$

In the formula, R_{t1} and R_{t2} are the retention time of one peak and next peak respectively; W_1 and W_2 are their peak width, respectively.

Results and Discussion

Effect of stationary-phase porosity

Dextran- and agarose-based porous media with four different porosities were used as the stationary phase. The experimental results were shown in Figure 3. The electrophoresis of anions in positive electric field was in the same direction as the hydrodynamic flow, thus CMP and UMP were accelerated by applying electric field. As small compounds, CMP and UMP are thought to be able to travel freely in the pore of Sephadex G-75, Sepharose 6B and 2B, but not in Sephadex G-10, because the exclusive range of Sephadex G-10 is in the same magnitude order as CMP and UMP. With the increase of the pore size of the media, larger pore volume led to longer travel distance of CMP and UMP, thus increased the discrimination of their retention times and the resolution between CMP and UMP. The most porous medium, Sepharose 2B, gave the best resolution, but gave wider peaks and more inferior bed stability. The rest experiments were made with Sepharose 6B.

Results without electric field application in Figure 3 showed that dextran- and agarose-based porous media had no ability to separate CMP and UMP. Interaction between the media and mononucleotides was assumed to be anionic repulsion (4) and hydrophilic interaction. Since CMP and UMP are negatively charged at pH 3.6, anionic repulsion between them hindered the adsorption of mononucleotides by the media, and the adsorption of mononucleotides by the media was not enough to separate them. When electric field was applied, the porous media performed another function reported in DNA electrophoresis system (5,6), that is, to prevent convective and diffusive effects of free-solution electrophoresis, thus to inhibit re-mixing of separated peaks. The electrophoresis in a chromatographic column could supply online detection and fractional collection. As was shown, higher resolution was expected with more porous medium when samples could travel more freely in this medium.

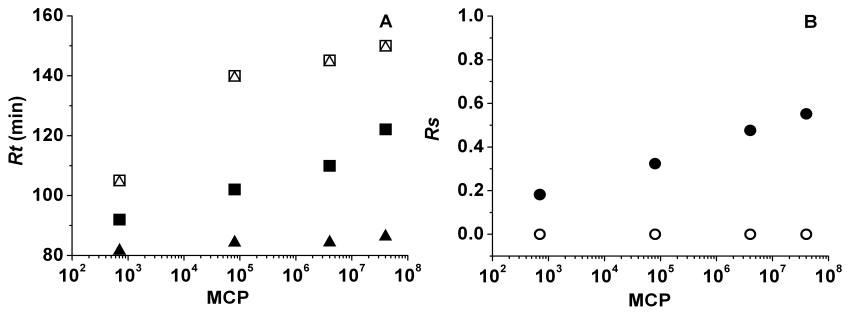

Figure 3. Logarithm of the Mr cut-off point (MCP) of the media vs. the solute retention time R_t (A) and resolution R_s (B) of CMP to UMP. R_t of CMP (■), UMP (▲), and R_s (●) were determined in electric field of 60 V/cm, and R_t of CMP (□), UMP (△), and R_s (○) were at the absence of electric field. Load: CMP (1.0 mg) and UMP (0.5 mg). Column: 40 × 0.6 cm i.d. Buffer: 2 mM NaOAc-HOAc buffer at pH 4.6. Flow rate: 0.19 mL/min without electric field; 0.18 mL/min in electric field of 60 V/cm.

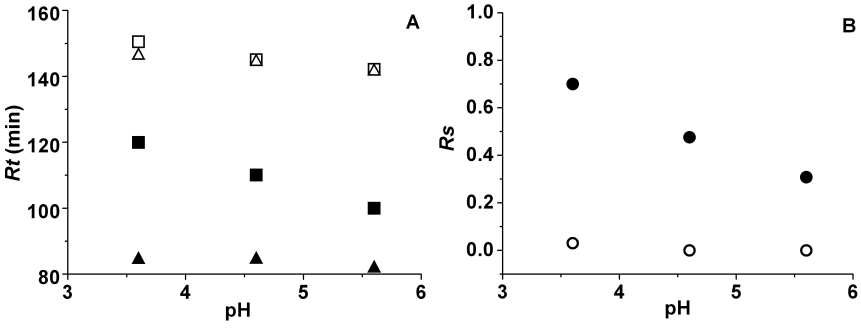

Figure 4. Buffer pH vs. R_t (A) and R_s (B) of CMP and UMP for Sepharose 6B. Rt of CMP (■), UMP (▲), and R_s (●) were determined in electric field of 60 V/cm, and R_t of CMP (□), UMP (△), and R_s (○) were at the absence of electric field. Other conditions were same as Figure 3.

Effect of buffer pH

The NaOAc-HOAc buffers at low concentration were used to assure low electric current and reduce Joule heat. Since the pKa value of pyrimidine group on CMP is 4.5, the effect of buffer pH (pH 3.6, 4.6, and 5.6) was evaluated. The results were shown in Figure 4. With the increase of buffer pH, the retention time of CMP obviously decreased, because its negative charge increased. The basicity of pyrimidine group on UMP is very little so that its retention time only decreased slightly with the increase of buffer pH. As a result, resolution between CMP and UMP decreased when buffer pH was enhanced. These revealed that pH 3.6 was the most proper buffer pH in this paper.

Effect of electric field modes

The separation in different electric field modes was studied. The results and recoveries were shown in Figure 5 and Table I.

When positive electric field was applied, UMP with higher electrophoretic mobility was much more accelerated, and the resolution was enhanced with the increase of electric field. However the recoveries of CMP and UMP (Nos. 4 and 5 in Table I) were dramatically diminished because they

might be electrolyzed at anode. This problem could be resolved by the shut-off of electric power before CMP or UMP reached the electrode (at 50 min). The result was shown in No. 6 in Table I.

When negative electric field was applied with cathode at the column outlet, buffer flow and electrophoresis of anions were in the opposite directions. It was observed that the electric field force on UMP was strong enough to counteract the buffer flow and draw UMP toward column inlet. Thus negative electric field was started at certain time after the sample loading and continued for 100 min, then was stopped (Nos. 1 and 2 in Table I). It was reported that DNA fragments taking negative charges could be electrically retained (5,6). Similarly, UMP taking negative charges could also been retained at certain flow rate. However, in buffer at pH 3.6, the pyrimidine group of CMP (p*K*a 4.5) is positively charged, thus the negative charges of CMP are less than UMP. CMP could not be retained as long as UMP with the accelerated flow rate aroused by electroosmotic flow (EOF) in the same direction as the liquid flow. As a result, the resolution was enhanced with higher electric field. Compared to the results in positive electric field, UMP and CMP could be completely separated at –120 V/cm electric field and got higher recoveries. On the contrary, the recovery of UMP was less than that at the absence of electric field. This phenomenon may result from the prolonged and broad peaks of the electrically retained UMP. The recovery of CMP could reach 94.8%, which was much higher than that at the absence of electric field. Without electric field at pH 3.6, the pyrimidine group of CMP may take positive charges, which induced less negative charges of CMP. Thus CMP could be partially adsorbed on the media and its recovery was smaller. When electric field was applied in either orientation, the recovery of CMP was dramatically increased. These phenomena revealed that electric field could enhance the elution of small compounds. According to electrostatic theory, there are diffusive electric double layers on the charged surfaces of agarose-based media. In the presence of electric field, solutes in the electrical double layer can move with the migration of the diffusive electric double layers (i.e., EOF) (22–26), which was observed as the alternation of the flow rate in the presence of electric field (Table I). The intraparticle diffusion resistance could be diminished and mass transfer could be enhanced. However UMP, taking more negative charges, is electrostatically repelled by gel. So the chromatographic recovery of UMP was as high as 84.3%. When negative electric field was applied, the liquid flow and EOF were counteracted with the electric field force on UMP. This might induce the prolonged and broad UMP peaks, similar as the phenomenon in reference (6), in which the electrical retention of DNA was observed. The effective way to improve the recovery and peak shape of the electrically retained sample was to enhance the conductivity of the buffer (6). However, too much Joule heat may prevent higher electric field, which was indispensable to retain mononucleotides.

Table I. Sample Recoveries with Different Electric Field and Applying Time

Trial No.	Condition: Electric field (V/cm)	Condition: Time course (min)*	Condition: Cor. flow (mL/min)†	Sample recovery (%): UMP	Sample recovery (%): CMP
1	–120	85–185	0.30	63.5	94.8
2	–60	70–170	0.22	62.4	96.3
3	0	–	0.19	84.3‡	33.4‡
4	60	0–150	0.18	21.8	26.5
5	120	0–150	0.17	6.0	12.4
6	120	0–50	0.17	40.3	60.8

* Electric field applying time course.
† Corresponding flow rate in the time course applying electric field.
‡ Sample recovery yield without electric field was calculated with the total sample amount in all fractionated liquid.

In this paper, RP-HPLC was used to determine sample concentrations in fractions. The retention times of CMP and UMP were 4.1 min and 4.6 min, respectively. The resolution was 0.95. So the sample amount for a RP column (4.6-mm i.d.) should be no more than 1 µg, which was far less than that by semi-preparative electrophoresis (1.5 mg for column of 6-mm i.d.).

In summary, a higher electric field is effective to enhance resolutions in either field orientation. Electrophoresis with positive electric field can result in shorter retention time, which is useful for fast separation. But the limited distance of the sample movement path may result in incomplete separation. Electrophoretic retain of the sample by negative electric field is also effective, especially for more chargeable samples. Higher resolution of them to non-charged samples can be achieved than electrophoresis in positive electric field, but longer time is needed.

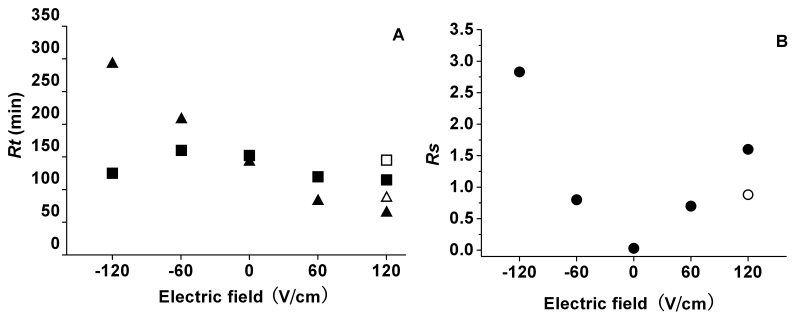

Figure 5. Effect of electric field in various modes on R_t (A) and R_s (B) of CMP and UMP. R_t of CMP (■), UMP (▲), and R_s (●) were resulted from Nos. 1~5 in Table I. R_t of CMP (□), UMP (△), and R_s (○) were resulted from No.6 in Table I. Medium: Sepharose 6B. Buffer: 2 mM NaOAc-HOAc buffer at pH 3.6. Flow rate: same as in Table I. Other conditions were same as Figure 3.

Column capacity with negative electric field

In this paper, resolution of mononucleotides by negative electric field of –120 V/cm was best. The column capacity in this electric field was determined by loading CMP and UMP with increased amount. Satisfactory separation was obtained at total nucleic acid loading up to 7.5 mg on a 40 × 0.6 cm i.d. column (bed volume 11.4 mL) (Figure 6C). The corresponding recoveries were 95.4% (CMP) and 64.0% (UMP). Increasing the sample loading beyond this level resulted in a loss of resolution and UMP appearance in CMP fraction. The capacity was much higher than reported capacity of 0.36 mg of DNA fractions with a 11 × 2.5 cm i.d. column (6).

When the load was increased, much higher electric current was observed. It was reported that the capacity of nucleic acids (DNA fragments) could be enhanced by the increase of the electric field (6,27) or by the increase of the conductivity of the buffer (6), but both methods would induce too much Joule heat. Therefore more effective cooling systems would be necessary for preparative purpose.

In the paper, retention time for Figure 6 was much longer than analytical chromatography. In reported DNA fractionation system (5), nucleic acids were fractionated by electrically retaining mechanism and the retention time was as long as 100–500 min. So the preparative electrophoresis needs long separation time.

As indicated in Figure 6, the retention time of the electrically retained solute UMP was determined by the mobile phase flow rate, the strength and application time of electric field, the EOF and the properties of other components. The mobile phase flow was driven by the liquid gravity, not by the pump at the outlet of the column, which merely transported mobile phase from the outlet electrode chamber to the UV detector. Thus the effect of the pump on the flow rate and the retention time was minor. Electric field is a key factor to affect the retention time. Increasing electric field could decrease the retention time, but the method would produce too much Joule heat and the heat problem has not been solved well. The time when the electric field was shut off is associated with the properties of other components in the sample. The larger the discrimination of their charge states is, the shorter the application period of the electric field and the retention time are.

The self-assembled apparatus mentioned in this paper is not automatic and must be run attentively. The semi-preparative method with the apparatus could present some effective results and give some proofs about the feasibility of this method. Further studies about the apparatus and the separation method are still in the making.

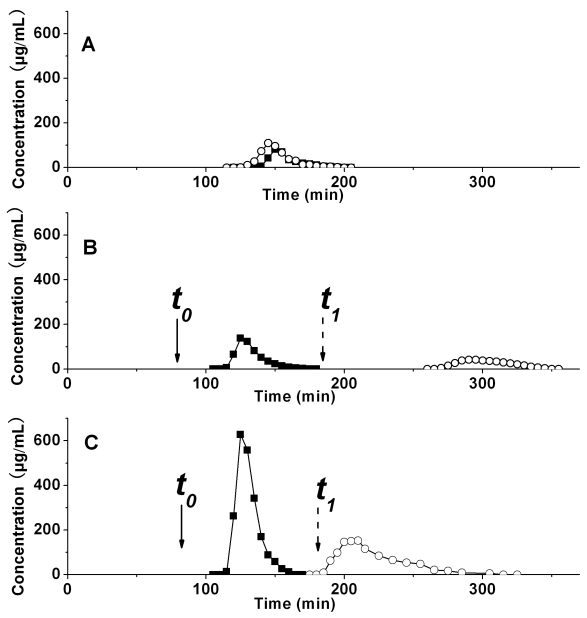

Figure 6. Chromatograms of CMP (■) and UMP (○). Load: (A)(B) CMP (1.0 mg) and UMP (0.5 mg); (C) CMP (5.0 mg) and UMP (2.5 mg). Medium: Sepharose 6B. Buffer: 2 mM NaOAc–HOAc buffer at pH 3.6. Flow rate: (A) 0.19 mL/min; (B) and (C) 0.30 mL/min. Electric field: (A) 0 V/cm; (B) and (C) –120 V/cm from t_0 (85 min) to t_1 (185 min), 6–8 mA.

Conclusions

The separation of mono- or oligonucleotides is always accomplished by ion-exchange chromatography in analytical or preparative scale. In this paper, a semi-preparative electrophoresis system with porous gels as the media was established to separate small polar nucleotides. The results indicated that electrophoresis in this system could separate small polar nucleotides in semi-preparative scale. There are some basic differences between typical ion-exchange chromatography and electrophoresis with negative electric field. First, when the electric field was stopped, the retained peak appeared very rapidly. Comparatively, elution from ion-exchange column needed the change of ionic strength or pH, which required more complicated equipment and more difficult control. The salt concentration in eluent was usually 20–100 mM, which was higher concentrated than several millimoles per liter in electrophoresis. The high salt concentration in eluate was not beneficial for the succeeding separation procedure. Secondly, the application of electric field could effectively enhance the mass transfer, so that more concentrated samples could be fractionated. Thirdly, the column capacity was not only decided by column dimensions as common chromatography, but also associated with the electric field and the buffer conductivity. Thus, an analytical column of 0.6-cm i.d. could prepare milligrams of compounds. It was illustrated that the application of a high electric field to porous media could separate small polar nucleotides or other chargeable small polar compounds in semi-preparative scale. Since separation of polar compounds from natural extracts (broth, combination chemistry, and phytochemistry) was limited in term of separation methodology (11), this electrokinetic method is a potential system in natural drug discovery.

Acknowledgements

Our work was supported by Shanghai Leading Academic Discipline Project, China (No. B203) and Shanghai Nanometer Project, China (No. 0259nm015).

References

1. S.R. Rudge, S.K. Basak, and M.R. Ladisch. Solute retention in electrochromatography by electrically induced sorption. *AIChE J.* **39:** 797–808 (1993).
2. S.K. Basak, A. Velayudhan, K. Kohomann, and M.R. Ladisch. Electrochromatographic separation of proteins. *J. Chromatogr. A* **707:** 69–76 (1995).
3. C. Keim and M. Ladisch. New system for preparatie electrochromatography of proteins. *Biotechnol. Bioeng.* **70:** 72–81 (2000).

4. C.M. Tellez and K.D. Cole. Preparative electrochromatography of proteins in various types of porous media. *Electrophoresis* **21:** 1001–1009 (2000).
5. K.D. Cole. Preparative concentration and size fractionation of DNA by porous media using a combination of flow and low electric field strength. *Biotechnol. Prog.* **13:** 289–295 (1997).
6. K.D. Cole, C.M. Tellez, and R.W. Blakesley. Separation of different physical forms of plasmid DNA using a combination of low electric field strength and flow in porous media: effect of different field gradients and porosity of the media. *Electrophoresis* **21:** 1010–1017 (2000).
7. S.M. Gryaznov. Oligonucleotide N3′-P5′ phosphoramidates as potential therapeutic agents. *Biochim. Biophys. Acta* **1489:** 131–140 (1999).
8. T. Da Ros, G. Spalluto, M. Prato, T. Saison-Behmoaras, A. Boutorine, and B. Cacciari. Oligonucleotides and oligonucleotide conjugates: a new approach for cancer treatment. *Curr. Med. Chem.* **12:** 71–88 (2005).
9. S. Shakeel, S. Karim, and A. Ali. Peptide nucleic acid (PNA)—a review. *J. Chem. Technol. Biotechnol.* **81:** 892–899 (2006).
10. J.F. Goossens, C. Foulon, A.-L. Villard, J.-Y. Puy, I. Lefebvre, C. Perigaud, C. Vaccher, and J.P. Bonte. Column selection and method development for the separation of nucleoside phosphotriester diastereoisomers, new potential anti-viral drugs. Application to cellular extract analysis. *Biomed. Chromatogr.* **19:** 415–425 (2005).
11. M.A. Strege. Hydrophilic interaction chromatography–electrospray mass spectrometry analysis of polar compounds for natural product drug discovery. *Anal. Chem.* **70:** 2439–2445 (1998).
12. H. Schlichtherle-Cerny, M. Affolter, and C. Cerny. Hydrophilic interaction liquid chromatography coupled to electrospray mass spectrometry of small polar compounds in food analysis. *Anal. Chem.* **75:** 2349–2354 (2003).
13. H. Zhang, Z. Guo, F. Zhang, Q. Xu, and X. Liang. HILIC separation of co-eluted flavonoids under RP-HPLC mode. *J. Sep. Sci.* **31:** 1623–1627 (2008).
14. L. Shi, G. Ying, Z. Tang, Y. Yi, J. Shan, and H. Liu. Separation of cytidine 5'-triphosphate biosynthesized from cytidine 5'-monophosphate on ion-exchange resin and HPLC analysis of cytidine compounds. *Appl. Biochem. Biotechnol.* **144:** 1–14 (2008).
15. P.R. Brown, C.S. Robb, and S.E. Geldart. Perspectives on analyses of nucleic acid constituents: the basis of genomics. *J. Chromatogr. A* **965:** 163–173 (2002).
16. T. Zhou and C.A. Lucy. Hydrophilic interaction chromatography of nucleotides and their pathway intermediates on titania. *J. Chromatogr. A* **1187:** 87–93 (2008).
17. H. Feng, N. Wong, S. Wee, and M.M. Lee. Simultaneous determination of 19 intracellular nucleotides and nucleotide sugars in Chinese Hamster ovary cells by capillary electrophoresis. *J. Chromatogr. B* **870:** 131–134 (2008).
18. B. Behnke and E. Bayer. Pressurized gradient electro-high-performance liquid chromatography. *J. Chromatogr. A* **680:** 93–98 (1994).
19. T. Tsuda. Electrochromatography using high applied voltage. *Anal. Chem.* **59:** 521–523 (1987).
20. L. Wittebolle, K. Verstuyft, W. Verstraete, and N. Boon. Optimisation of the amino–carboxy coupling of oligonucleotides to beads used in liquid arrays. *J. Chem. Technol. Biotechnol.* **81:** 476–480 (2006).
21. L. Feng and F. Zhao. Preparative electrochromatography of tea polyphenols and caffeine. *Chromatographia* **69:** 1–8 (2009).
22. Z. Liu, G. Yin, S. Feng, D. Wang, F. Ding, and N. Yuan. Oscillatory electroosmosis-enhanced intra/inter-particle liquid transport and its primary applications in the preparative electrochromatography of proteins. *J. Chromatogr. A* **921:** 93–98 (2001).
23. G. Tan, Q. Shi, and Y. Sun. Oscillatory transverse electric field enhances mass transfer and protein capacity in ion-exchange electrochromatography. *J. Chromatogr. A* **1098:** 131–137 (2005).
24. G. Jia, X. Dong, and Y. Sun. Dye-ligand affinity electrochromatography with transverse and/or longitudinal electric field. *Sep. Purif. Technol.* **59:** 277–285 (2008).
25. A.I. Liapis and B.A. Grimes. Film mass transfer coefficient expressions for electroosmotic flows. *J. Colloid Interface Sci.* **229:** 540–543 (2002).
26. G.F. Chen and U. Tallarek. Effect of intraparticle porosity and double layer overlap on electrokinetic mobility in multiparticle systems. *Langmuir* **19:** 10901–10908 (2003).
27. Y.G. Park. Separation of DNA by mass-transfer properties in the presence of an electric field. *Biochem. Eng. J.* **7:** 213–221 (2001).

Manuscript received May 26, 2009;
revision received January 29, 2010.