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DNA sequencing by capillary electrophoresis using mixtures of polyacrylamide and poly(N,N-dimethylacrylamide)

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Abstract

The possibility of using polymer mixtures with different chemical compositions as a DNA sequencing matrix by capillary electrophoresis (CE) has been exploited. Polyacrylamide (PAM, 2.5%, w/v) having a molecular mass of $2.2 \cdot 10^6$ has been mixed with poly(N,N-dimethylacrylamide) (PDMA) having molecular masses of 8000, 470 000 and $2.1 \cdot 10^6$ at concentrations of 0.2, 0.5 and 1% (w/v). Unlike polymer mixtures of the same polymer with different molecular masses, the use of polymer mixtures with different chemical compositions encounters an incompatibility problem. It was found that the incompatibility increased with increasing PDMA molecular mass and PDMA concentration, which resulted in decreased efficiency in DNA sequencing. Also, the incompatibility had a more pronounced effect on the efficiency as the base number was increased. However, by choosing a low-molecular-mass PDMA of 8000 and a low concentration of 0.2% (w/v), the incompatibility of PAM and PDMA has been alleviated. At the same time, the advantage of using polymer mixtures revealed a higher efficiency for such a polymer mixture when compared with PAM. The mixture also endowed the separation medium with a dynamic coating ability. An efficiency of over $10 \cdot 10^6$ theoretical plates per meter has been achieved by using the bare capillaries without the additional chemical coating step.

Keywords: Sequencing methods; DNA; Polyacrylamide; Polydimethylacrylamide

1. Introduction

The human genome project (HGP) [1] has stimulated dramatic improvements in DNA sequencing technology. An automated DNA sequencer using capillary electrophoresis (CE) with multiple capillaries and replaceable polymer solutions has been proven to be the most feasible approach to achieve the final goal [2]. However, significant improvements in DNA sequencing technology are still required for biological and medical research in the future, and should remain a high-priority commitment of the HGP [3].

Currently, most investigations on DNA sequencing by CE have been focused on the instrumentation of capillary array electrophoresis (CAE) in order to make it more and more powerful for large-scale DNA sequencing analysis [4–11]. Recently, capillary array electrophoresis in micro-fabricated chips [12–16] has attracted more attention because it promises to use a smaller amount of reagent, produce more condensed capillary array and integrate DNA preparation and sequencing procedures together as a whole. In contrast, fewer investigations on the

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improvement of separation media for DNA sequencing analysis by CE have appeared [17].

Polyacrylamide (PAM) is possibly the most successful separation medium investigated for DNA sequencing analysis by CE. By using this polymer, DNA sequencing analyses of up to 1000 bases in less than 1 h [18] and 1300 bases in 2 h [17] have been reported by Karger and co-workers. In addition to PAM, other polymers have also been investigated, with polyethylene oxide (PEO) achieving a read length of 1000 bases in about 7 h [19], poly(N,N-dimethylacrylamide) (PDMA) about 600 bases in about 2 h [20], hydroxyethylcellulose (HEC) greater than 500 bases in about 1 h [21], and other polymers smaller than 500 bases. Although good separation ability is the highest priority in selecting a polymer for a specific separation medium for CAE, dynamic coating ability to the fused-silica capillary inner surface can be considered an added plus since it can greatly facilitate the automation of CAE. Madabhushi [20] has investigated the dynamic coating ability of commonly used polymers in DNA sequencing analysis and concluded that PDMA has the best coating ability and PAM the worst. Thus, a compromise could be in order if the properties of PAM and PDMA can be combined.

We have successfully combined the good separation ability of PAM and the good coating ability of PDMA in DNA sequencing by using random copolymers of AM and DMA [22]. Unfortunately, the copolymer showed higher viscosity than PAM. In addition, it is difficult to synthesize very high-molecular-mass copolymers by microemulsion polymerization as Goetzinger et al. have done in the synthesis of PAM [23] due to the instability of the microemulsions induced by the hydrophobic DMA. However, the use of polymer mixtures may serve the same purpose.

Polymer mixtures of PEO have been used first by Yeung and co-workers for the separation of both double-stranded DNA (dsDNA) [24] and single-stranded DNA (ssDNA) [19,25] by CE. Their experiments showed that low-molecular-mass polymers provided higher efficiency for smaller DNA fragments, and vice versa [24]. Polymer mixtures were beneficial to the separation of a broad range of DNA fragments. Since then, polymer mixtures of HEC [26] and of HEC with agarose [27] have been successfully used for dsDNA separation. In 1996, we reported the use of PAM mixtures for DNA sequencing analysis [28]. The addition of a small amount of low-molecular-mass PAM in the very high-molecular-mass PAM matrix has been employed by Karger and co-workers to achieve long read length DNA sequencing within short run times without a significant increase in viscosity and thereby in migration time [17,18].

The use of polymer mixtures with different chemical compositions has never been successful for DNA separation. Both HEC and agarose were basically modified polysaccharides and should, therefore, not be counted as polymer mixtures with different chemical compositions. However, we did take advantage of the concept of interpenetrating networks of polyacrylamide and polyvinylpyrrolidone [29]. Kim and Yeung [19] tried to use a mixture of PEO and hydroxypropylcellulose (HPC) for DNA sequencing analysis and found the separation to be very poor. The failure was attributed to the incompatibility of the two polymers. In this study, the possibility of incorporating PDMA in a high-molecular-mass PAM matrix for DNA sequencing analysis is investigated. It was found that the good separation ability of PAM and the good dynamic coating ability of PDMA could be combined in a polymer mixture by careful selection of the molecular mass and the concentration of PDMA. Higher efficiency could also be retained in the polymer mixture.

2. Experimental

2.1. Chemicals

Acrylamide, ammonium persulfate (APS), tris-(hydroxymethyl)aminomethane (Tris), N-tris-(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma (St. Louis, MO, USA), and N,N,N′,N′-tetramethylenediamine (TEMED), *n*-butanol, *tert.*-butanol, benzene, hexanes, methylene chloride, acetone, formamide and hydrochloric acid from Fisher Scientific (Pittsburgh, PA, USA). N,N′-Dimethylacrylamide was purchased from Polysciences (Warrington, PA, USA) and distilled under vacuum before use. 2,2-Azobis(2-

methylpropionitrile) (AIBN) was purchased from Spectrum (Gardena, CA, USA) and recrystallized twice from ethanol. The electrophoresis buffers were 1×TTE (50 m*M* Tris–50 m*M* TAPS–2.0 m*M* EDTA) for the anode and 1×TTE–5 *M* urea for the cathode. The separation matrix used a 1×TTE–5 *M* urea buffer. Water used in all reactions and solutions was deionized (18.2 MΩ) with a Milli-Q water purification system (Millipore, Worcester, MA, USA).

2.2. Preparation of polymers

2.2.1. PAM

PAM with a molecular mass of $2.2 \cdot 10^6$ was synthesized by polymerization of a 0.1 g/ml AM aqueous solution [that was bubbled with ultra-high-purity (UHP) helium for 5 h to eliminate dissolved oxygen before polymerization] at 1°C for 24 h using 0.09 μl/ml TEMED and 0.09 mg/ml APS as initiators. The reaction product was diluted with water to 0.008 g/ml, precipitated by acetone (1:4 times volume of water), and then dried under vacuum.

2.2.2. PDMA I

PDMA with a molecular mass of 8000 was synthesized by polymerization of a 0.1 g/ml DMA solution in *n*-butanol (that was bubbled with UHP helium for 1 h to eliminate dissolved oxygen before polymerization) at 70°C for 4 h using 1 mg/ml AIBN as an initiator. The reaction product was distilled to dry, dissolved in benzene (0.05 g/ml), precipitated by hexane (two times volume of benzene), and then dried under vacuum.

2.2.3. PDMA II

PDMA with a molecular mass of 470 000 was synthesized by polymerization of a 0.7 g/ml DMA solution in *t*-butanol [that was bubbled with UHP helium for 1 h to eliminate dissolved oxygen before polymerization] at 55°C for 15 min using 0.82 mg/ml AIBN as an initiator. The reaction product was dissolved in methylene chloride (0.035 g/ml), precipitated by hexane (1.75 times volume of methylene chloride), and then dried under vacuum.

2.2.4. PDMA III

PDMA with a molecular mass of $2.1 \cdot 10^6$ was synthesized by polymerization of a 0.14 g/ml DMA aqueous solution (that was bubbled with UHP helium for 1 h to eliminate dissolved oxygen before polymerization) at 25°C overnight using 0.4 μl/ml TEMED and 0.4 mg/ml APS as initiators. The reaction product was diluted with water to 0.015 g/ml, precipitated by acetone (four times volume of water), and then dried under vacuum.

To prepare a separation medium, 1×TTE–5 *M* urea buffer and PDMA solutions in 1×TTE–5 *M* urea buffer with a different molecular mass and concentration were first prepared. Then the required volume of 1×TTE–5 *M* urea buffer or PDMA solution was added to a known mass of PAM dry polymer to 2.5% (w/v). After the PAM was swollen by the buffer overnight, the solution was vortexed for 30 s, twice a day with at least a 6-h interval. After 2–3 days, the solution was degassed by ultracentrifugation ($7 \cdot 10^4$ *g*) before use.

2.3. Sequencing chemistry

Sequencing reactions were performed using an ABI Prism Dye primer (−21 M13 forward) cycle sequencing ready reaction kit with AmpliTaq® DNA polymerase, FS (PE Biosystems/Perkin-Elmer, Foster City, CA, USA) on a pGEM3Zf(+) double-stranded template. Single dye-labeled sequencing reactions were performed using the FAM labeled primer and the C termination mix. The DNA sequencing was carried out with a GeneAmp PCR system 2400 (PE Biosystems/Perkin-Elmer) using the following cycling conditions: 15 cycles of 10 s at 95°C, 5 s at 50°C and 1 min at 70°C, followed by 15 cycles of 10 s at 95°C and 1 min at 70°C. The reaction products were purified by ethanol precipitation followed by two washes with 75% (v/v) ethanol and resuspended in 20 μl deionized formamide.

2.4. Laser-induced fluorescence detection

A water-cooled Ar-ion laser was used to generate an excitation beam at $\lambda = 488$ nm and an incident power of about 5 mW. The laser beam was focused by a lens with a 25-cm focal length, reflected by a

dichroic mirror (550DRLP, Omega Optical, Brattleboro, VT, USA), and focused again by using a 10× objective to a spot within the separation channel. The fluorescence was collected by the objective, passed through the dichroic mirror, and filtered by a band-pass filter (530DF30, Omega Optical) to the photomultiplier tube (PMT) (Model RFI/B-293F, Thorn EMI Electron Tubes Middlesex, UK). In order to get images of the capillary, a charge coupled device (CCD) camera (SSC-M350, Sony, New York, NY, USA) was also connected to the set-up. A white beam from the illuminator on the microscope illuminated the capillary. It was then focused by the objective, reflected by a slide in-and-out mirror, magnified by a Zoom 6000 System (D.O. Industries, Rochester, NY, USA) and then detected by the CCD camera. The microscope (Karl Zeiss, Melville, NY, USA) and the CCD camera provided a means to achieve good optical quality and to obtain a fast alignment.

2.5. CE procedures

Fused-silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) 50 cm×75 μm I.D.×365 μm O.D. were used. A detection window was opened at 10 cm from the anodic end by stripping off the polyimide coating with a razor blade. For sequencing with PAM as a separation medium, the inner wall of the capillary was covalently coated with PAM using the protocol described by Hjerten [30]. For sequencing with the polymer mixtures, a capillary was simply washed with 1 *M* HCl for 10 min. The separation medium was filled into the capillary tubing by using a gas tight syringe. Before each electrophoresis run, the capillary column was conditioned under an electric field strength of 300 V/cm until the current became stable (generally about 10 min). The DNA sample was electrokinetically injected into the capillary at an electric field strength of 300 V/cm for 5 s. Electrophoresis was conducted under an electric field strength of 150 V/cm.

2.6. Characterization of the polymers

The intrinsic viscosity of PAM and PDMA in water was measured with an Ubbelohde viscometer at 25°C. The plot of reduced viscosity (η_{sp}/C) versus concentration (C) was extrapolated to zero concentration, and the intercept yielded the intrinsic viscosity. M_v of PAM was calculated to be $2.2\cdot10^6$ using the Mark–Houwink equation ($[\eta] = kM_v^a$, where $k = 6.5\cdot10^{-3}$ ml/g, $a = 0.82$ in water at 30°C [31]). M_v of PDMA I, II and III were calculated to be 8000, 470 000 and $2.1\cdot10^6$ by using the Mark–Houwink equation with $k = 0.023$ ml/g, $a = 0.72$ in water at 30°C [32].

3. Results and discussion

The separation conditions, which had been optimized to achieve longer read length, were derived from the previous investigation on the use of copolymers of AM and DMA as a separation media for DNA sequencing by CE. They were 50 cm (effective capillary length 40 cm)×75 μm capillary I.D., 300 V/cm for 5 s injection and 150 V/cm for separation. Under these separation conditions, four separation media: 2.5% (w/v) PAM (molecular mass $2.2\cdot10^6$), 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA (8000), 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA (470 000) and 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA ($2.1\cdot10^6$) were tested for the sequencing of the polymerase chain reaction (PCR) products prepared with FAM-labeled −21M13 forward primer on pGEM3Zf(+) and terminated with ddCTP. The selection of a relatively low concentration of PDMA was to alleviate the expected incompatibility problem.

In order to make a comparison, the resolution of ten pairs of fragments with lengths of 45–46, 158–159, 216–217, 261–262, 289–290, 357–358, 438–439, 532–533, 616–617, and 698–699 bases was calculated by using the equation of $R_s = 2(t_2 - t_1)/[1.699(w_2 + w_1)]$, where R_s, t and w are resolution, migration time and peak width at half height, respectively. The results are depicted in Fig. 1 as a plot of resolution versus base number. As seen in Fig. 1, an increase in the PDMA molecular mass in the PAM–PDMA mixtures resulted in a decrease in resolution except for the 45–46 fragments pair. This result is different from that reported recently by Karger and co-workers [17], where 2% (w/w) PAM (molecular mass $10\cdot10^6$)–0.5% (w/w) PAM (270 000) was

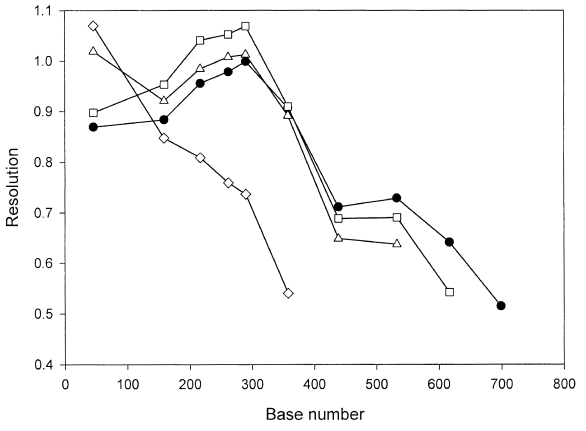

Fig. 1. Resolution vs. base number using PAM and PAM–PDMA mixtures as separation media for DNA sequencing by CE: effect of the molecular mass of PDMA. Filled circles: 2.5% (w/v) PAM ($2.2\cdot10^6$); open squares: 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA (8000); open triangles: 2.5 % (w/v) PAM ($2.2\cdot10^6$)–0.5 % (w/v) PDMA (470 000); open diamonds: 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA ($2.1\cdot10^6$). Separation conditions: 50 cm (40 cm effective length)×75 μm I.D.; electrokinetic injection at 300 V/cm for 5 s; electrophoretic separation at 150 V/cm.

found to achieve significantly better resolution than 2% (w/w) PAM ($10\cdot10^6$)–0.5% (w/w) PAM (50 000). In comparison with PAM, the addition of PDMA resulted in an increase in resolution for smaller DNA fragments and a decrease in resolution for larger DNA fragments. As the molecular mass of PDMA was increased, the cross point of the resolution curve for the mixture relative to that for PAM moved to lower base numbers. In order to examine these effects in detail, selectivity and efficiency were then calculated and compared.

As suggested by Carrilho et al. [33], there are two parameters, selectivity and efficiency, affecting resolution with relationships: $R_s = (SN^{1/2})/4$, where S and N are selectivity and efficiency, respectively. The selectivity and separation efficiency can be calculated by the equations $S = 2(t_2 - t_1)/(t_2 + t_1)$ and $N = 5.54(t/w)^2$. Our calculation showed that the selectivity of 2.5% (w/v) PAM ($2.2\cdot10^6$) and 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA (8000) were almost the same for the ten pairs of fragments, possibly due to the very low molecular mass of PDMA. However, as the PDMA molecular mass was increased in the mixtures, the selectivity also increased, which was more obvious for smaller DNA fragments and leveled off for longer DNA fragments (data not shown). This result is basically the same as reported by Zhou et al. [17]. The difference was found in efficiency, which was also the case for 2% (w/v) PAM ($10\cdot10^6$)–0.5% (w/w) PAM (270 000) and 2% (w/w) PAM ($10\cdot10^6$)–0.5% (w/w) PAM (50 000) [17], but in contrast, the efficiency decreased for the PAM–PDMA mixture as the molecular mass of PDMA was increased, as shown in Fig. 2. This could be caused by the expected incompatibility of the two polymers. Our results suggested that the incompatibility increased with increasing molecular mass of PDMA. The incompatibility was so significant that it overwhelmed the increased selectivity and resulted in a decrease in resolution except for the 45–46 fragment pair. There might be another reason for the drop in the efficiency with increasing molecular mass of PDMA, i.e. hydrophobic interactions of ssDNA with the hydrophobic PDMA. However, since PDMA can also provide very good separation of ssDNA [20], the effect was considered insignificant.

It is important to note that in comparison with

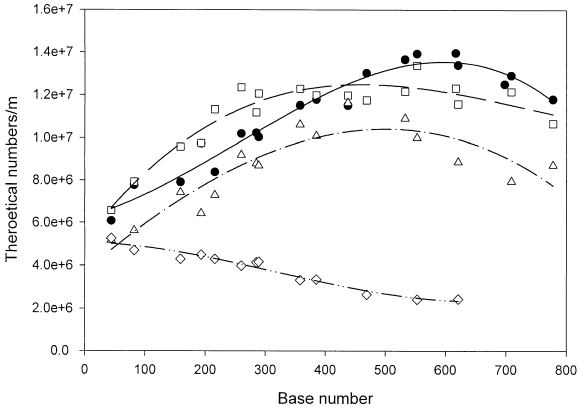

Fig. 2. Efficiency vs. base number using PAM and PAM–PDMA mixtures as separation media for DNA sequencing by CE: effect of the molecular mass of PDMA. In order to guide the eyes, a cubic function ($y = y_0 + ax + bx^2 + cx^3$) was used to fit the experimental values of theoretical plate number vs. base number. Filled circles with solid line: 2.5% (w/v) PAM ($2.2\cdot10^6$); open squares with long dash line: 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA (8000); open triangles with dash–dot line: 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA (470 000); open diamonds with dash–dot–dot line: 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA ($2.1\cdot10^6$). Separation conditions as in Fig. 1.

PAM, the addition of 0.5% (w/v) PDMA with a molecular mass of 8000 resulted in an increase in efficiency for smaller DNA fragments and a decrease in efficiency for larger DNA fragments. It tells us that the advantage of using polymer mixtures has been retained in terms of higher efficiency. However, this advantage has been overwhelmed by the polymer incompatibility for larger DNA fragments. As there was no significant difference in selectivity for these two separation media, the resolution difference came mainly from the difference in efficiency. In contrast, the resolution difference between 2.5% (w/v) PAM ($2.2\cdot10^6$) and 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA (470 000), or 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.5% (w/v) PDMA ($2.1\cdot10^6$) was caused by combined effects of selectivity and efficiency.

The effect of PDMA concentration on the separation was then investigated. In order to alleviate the incompatibility effect of PDMA and PAM, the lowest molecular mass of 8000 was tested at three different concentrations: 0.2, 0.5 and 1% (w/v). The plot of resolution versus base number is demonstrated in Fig. 3. An increase in the PDMA concentration of the PAM–PDMA mixture resulted in a decrease in resolution, indicating that the incompatibility of the two polymers became stronger. It is important to note that in comparison with 2.5% (w/v) PAM ($2.2\cdot10^6$), 2.5% (w/v) PAM ($2.2\cdot10^6$)–0.2% (w/v) PAM (8000) produced a higher resolution throughout the electropherogram, although the improvement became smaller for fragments greater than 600 bases.

In order to examine the concentration effect in detail, selectivity and efficiency were then calculated and compared. According to our calculation, the selectivity of 2.5% (w/v) PAM ($2.2\cdot10^6$) and 2.5% (w/v) PAM ($2.2\cdot10^6$)–PDMA (8000) with concentrations of 0.2, 0.5 and 1% (w/v) were almost the same for the ten pairs of fragments (data not shown), possibly due to the very low molecular mass of PDMA. However, we did observe the sequencing size limit of these separation media, which appeared as a big comigration peak of large DNA fragments at the end of the electropherograms, decreased slightly with increasing PDMA concentration. This change should be caused by the decrease in selectivity for large DNA fragments with increasing PDMA concentration, as reported by Salas-Solano et al. [18]. Hence, the resolution change was mainly caused by the change in efficiency as illustrated in Fig. 4. With PDMA concentration as low as 0.2% (w/v), the incompatibility of the two polymers was no longer

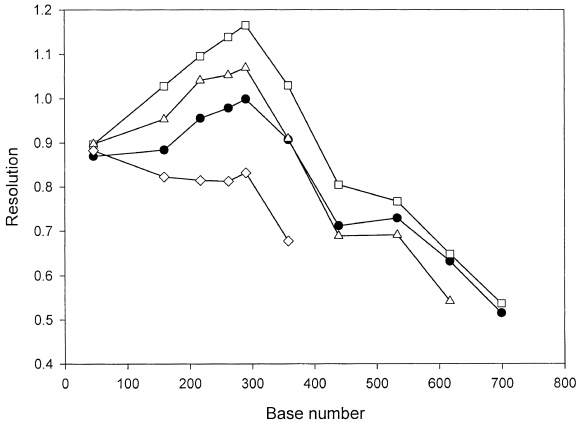

Fig. 3. Resolution vs. base number using PAM ($2.2\cdot10^6$) and PAM ($2.2\cdot10^6$)–PDMA (8000) mixtures as separation media for DNA sequencing by CE: effect of PDMA concentration. Filled circles: 0% (w/v); open squares: 0.2% (w/v); open triangles: 0.5% (w/v); open diamonds: 1.0% (w/v). Separation conditions as in Fig. 1.

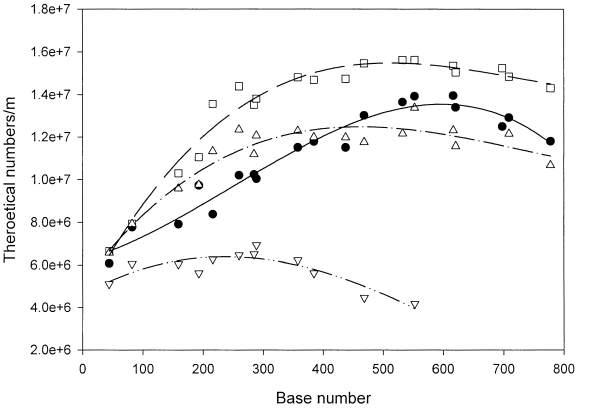

Fig. 4. Efficiency vs. base number using PAM ($2.2\cdot10^6$) and PAM ($2.2\cdot10^6$)–PDMA (8000) mixtures as separation media for DNA sequencing by CE: effect of PDMA concentration. In order to guide the eyes, a cubic function ($y = y_0 + ax + bx^2 + cx^3$) was used to fit the experimental values of theoretical plate number vs. base number. Filled circles with solid line: 0% (w/v); open squares with long dash line: 0.2% (w/v); open triangles with dash–dot line: 0.5% (w/v); open diamonds with dash–dot–dot line: 1.0% (w/v). Separation conditions as in Fig. 1.

important and the advantage of using polymer mixtures showed up as an increase in efficiency. With increasing PDMA concentration, the incompatibility of the two polymers became more important so that it overwhelmed the advantage and ruined the separation.

The increased efficiency with the PAM–PDMA mixture might be explained by the theory of network dynamics proposed by Cottet et al. [34]. Network dynamics reflected by the rate of dissociation of entanglement can be expressed as the lifetime of the pores or the network relaxation time. A slowdown in network relaxation time can increase the separation efficiency. Since PDMA and PAM are basically incompatible, the addition of PDMA to the PAM solution might increase the relaxation time of the PAM network, and result in an increase in efficiency. However, as the molecular mass or the concentration

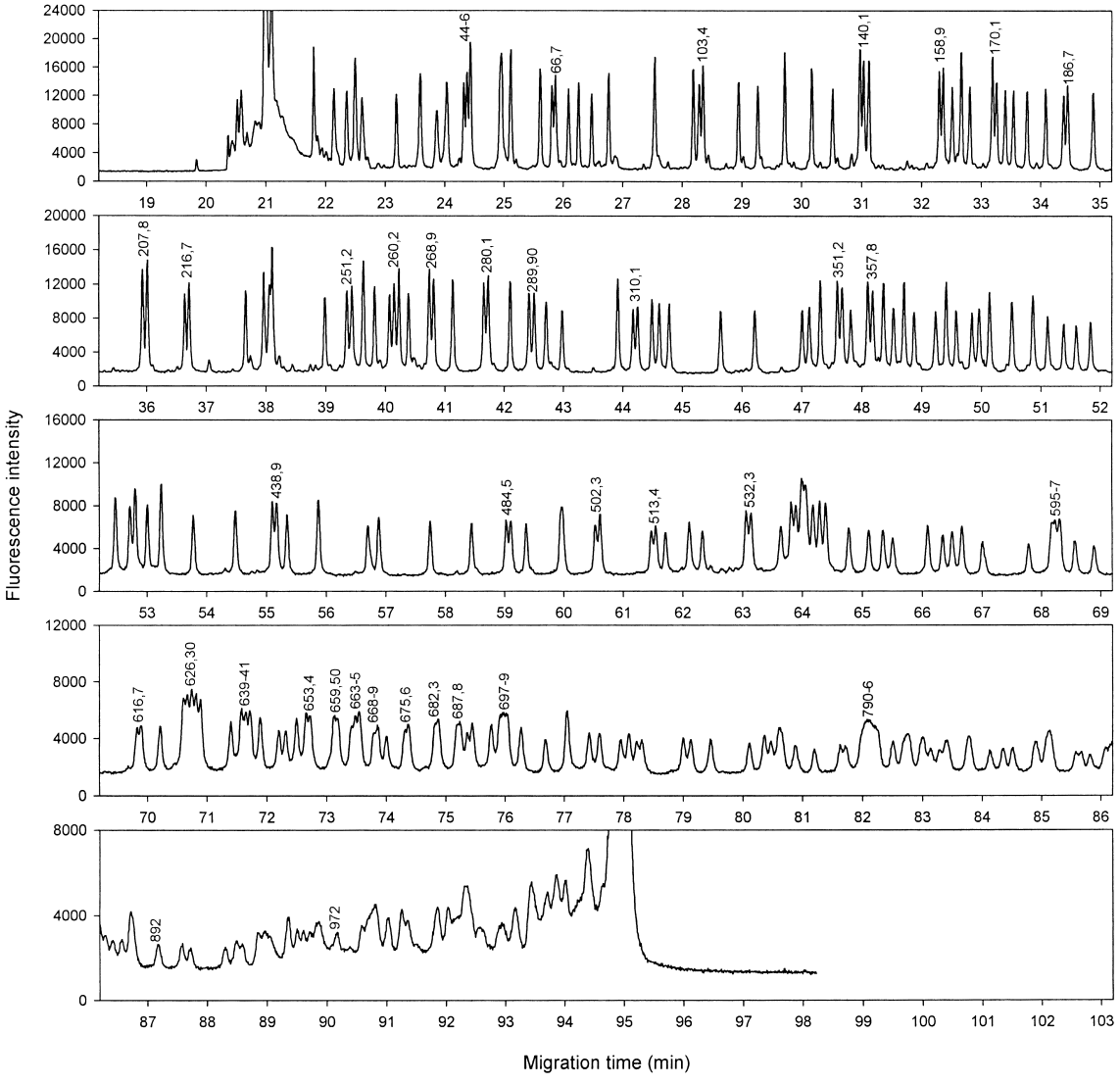

Fig. 5. DNA sequencing by CE using 2.5% (w/v) PAM ($2.2 \cdot 10^6$)–0.2% (w/v) PDMA (8000) as a separation medium. Separation conditions were as in Fig. 1. The DNA sample was prepared with FAM-labeled −21M13 forward primer on pGEM3Zf(+) and terminated with ddCTP. The separation has been achieved by using bare capillaries without an additional chemical coating step.

of PDMA was increased, local aggregates of each type of polymer might form. This incompatibility could then result in a non-homogeneous polymer–DNA interaction and decrease the efficiency in DNA sequencing.

The effect of PDMA concentration on the separation was also investigated with molecular masses of 470 000 and $2.1 \cdot 10^6$. The results showed the same tendency, i.e. higher PDMA molecular mass and concentration caused higher incompatibility of PAM and PDMA, and therefore lower efficiency in DNA sequencing.

In conclusion, using polymer mixtures could combine the advantages of different polymers in DNA sequencing by CE. However, extreme care should be taken to alleviate the incompatibility problem of the two polymers. Our results showed that the incompatibility of the two polymers decreased with decreasing polymer concentration and molecular mass. By incorporating low concentrations of low-molecular-mass PDMA in high-molecular-mass PAM solution, good dynamic coating ability of PDMA and good separation ability of PAM has been successfully combined. The advantage of using polymer mixtures also showed up as a higher separation efficiency ($10 \cdot 10^6$ theoretical plates per meter has been achieved by using the bare capillaries without the additional chemical coating step). The use of 2.5% (w/v) PAM ($2.2 \cdot 10^6$)–0.2% (w/v) PDMA (8000) for sequencing of the PCR reaction products prepared with FAM-labeled −21M13 forward primer on pGEM3Zf(+) and terminated with ddCTP are illustrated in Fig. 5. One base resolution of 0.5 up to 730 bases has been achieved within 80 min at ambient temperatures. Further studies with this mixture for four-color DNA sequencing at elevated temperatures are under investigation. Elevated temperature is required by DNA sequencing to suppress ssDNA compression. It has been shown that elevated temperature also increases the read length in DNA sequencing by CE using PAM as a separation medium [17,18]. However, it should also affect the PDMA dynamic coating, which is under investigation.

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