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Electrophoresis of DNA in novel thermoreversible matrices

We demonstrate the feasibility of temperature-sensitive polymers as novel matrices for capillary and slab electrophoresis of DNA. These matrices combine the ease-of-use of agarose with resolution properties of polyacrylamide. Two classes of matrices are used: (i) aqueous suspensions of gel microspheres and (ii) solutions containing uncross-linked temperature-sensitive polymers. When heated, the viscosity of these solutions drops dramatically because of the phase transition behavior of these polymers, and therefore these formulations are easy to pour or load. Results are presented for separation of double-stranded DNA fragments (< 2000 bp) in capillary, tube, and slab electrophoresis. Preliminary results are also presented for separation of single-stranded sequencing fragments by capillary electrophoresis. In the tube format, good resolution was obtained for ΦX174/*Hae*III fragments. In the slab format, excellent resolution of double-stranded DNA was obtained, including simultaneous separation of a 10 bp ladder up to 150 base pairs. In the capillary format, pBR322/*Msp*I fragments were completely resolved, and single-base resolution of sequencing fragments was obtained up to 150 bases. We believe that temperature-sensitive polymers represent a new and promising class of electrophoretic media.

1 Introduction

1.1 Slab gel media

The two most common separation media for slab gel electrophoresis of DNA continue to be agarose and polyacrylamide. Derived from seaweeds, agarose is a popular and convenient matrix because gels can be prepared by the 'heat-and-pour' method. Agarose gels are effective at separating DNA fragments from tens to millions of base pairs. However, resolution of small, double-stranded (ds) DNA fragments in agarose gels is inferior to that obtained in polyacrylamide gels. For that reason, cross-linked polyacrylamide is used for fine resolution of DNA fragments smaller than 2000 bp; larger DNA fragments are not well resolved [1]. While it is straightforward, in principle, to prepare polyacrylamide gels, in practice the polymerization is prone to irreproducibility because free-radical solution polymerization is highly dependent on variables that are often not controlled rigorously, such as temperature and oxygen content. Furthermore, the gel casting apparatus often leaks, potentially exposing the user to neurotoxic acrylamide monomer.

Considering the importance and widespread use of electrophoresis today, alternate matrices for electrophoresis have been relatively understudied. Recently, Chiari and Righetti [2] presented an excellent review of new types of separation matrices for slab electrophoresis. These matrices are prepared by the user by polymerizing novel monomers or novel monomer/polymer mixtures, and therefore suffer from the drawbacks in gel preparation associated with cross-linked polyacrylamide gels. We refer to the type of electrophoresis gel exemplified by cross-linked polyacrylamide as a 'chemical' gel [3] because the topology of the network is determined by the chemical reaction of a bifunctional cross-linked agent (often N,N'-methylenebisacrylamide) with growing chains of a monofunctional monomer. Thus the structure is fixed by covalent bonding. The network is irreversible because it cannot be converted to a free-flowing solution without chemical degradation. In contrast, agarose belongs to the class of electrophoresis gels we call 'physical' gels [3] because the network is formed by noncovalent interactions. Powdered, underivatized agarose is not soluble in aqueous buffers at room temperature, but dissolves easily at high temperatures to form a clear, transparent solution. The solution can be poured (generally at 50–60 °C) into a casting tray and is allowed to cool and set, whereupon the gel also opacifies slightly. As a heated agarose solution cools, agarose chains hydrogen-bond to form double-helical structures, which in turn aggregate to form a three-dimensional network. Heating an agarose gel breaks these interchain hydrogen bonds and returns the network to a solution state; therefore, an agarose gel is also 'reversible'.

1.2 Capillary electrophoresis media

It is difficult to prepare uniform, homogeneous, bubble-free, and stable gel-filled capillaries. Furthermore, convection and diffusion have a much lower effect on band broadening in a capillary than in a slab gel. Therefore, the separation matrix does not need to be a rigid gel and, instead, solutions of uncross-linked polymers have been used successfully as replaceable matrices for capillary electrophoresis of double-stranded and single-stranded DNA. Bode *et al.* [4] pioneered the use of

uncross-linked polymers in electrophoresis on cellulose acetate membranes, and Zhu *et al.* [5] and Chin *et al.* [6] later extended the application to CE. Many types of uncross-linked polymers have been used for capillary electrophoresis of DNA, including polyacrylamide [7–11], cellulose derivatives [8, 12–18], polyethylene glycol [13, 19], dextran [20], and glucomannan [21]. The use of uncross-linked polymers is attractive in capillary electrophoresis because, in principle, a polymer solution can be pumped in and out of a capillary, allowing the capillary to be used many times. In practice, however, polymer solutions that provide high resolution are also viscous; the loading of capillaries therefore requires long times or high pressure.

1.3 Temperature-sensitive polymeric media for electrophoresis

Here, we describe novel types of electrophoresis gels based on temperature-sensitive polymers. Our goal is to combine high resolution and thermoreversibility in a single matrix. In this work, we describe the use of temperature-sensitive polymers as electrophoretic media for slab, tube, and capillary electrophoresis of DNA fragments. These prepolymerized* matrices are unique because they offer polyacrylamide-like resolution properties and are easy to prepare and use.

1.3.1 Theoretical background of lower consolute solution temperature (LCST) polymers and gels

Polymer solubility and gel swelling in aqueous solutions depends on a number of solution variables. The set of solution variables which influence solubility and swelling significantly depends on specific polymer chemistry. Even polyacrylamide gel slabs shrink or swell with changes in temperature or solution composition [22]. Hydrogels which undergo large changes in swelling in response to solution conditions are termed environmentally sensitive or 'intelligent' hydrogels. Over four decades ago, Katchalsky [23] studied extensively the large volume changes in polyelectrolyte gels which occur with changes in pH. In recent years, environmentally sensitive hydrogels have been investigated for potential applications in biotechnology, medicine, chemomechanical systems, and other fields [24–30].

In this work, we investigate temperature-sensitive polymers that are characterized by lower consolute solution temperature (LCST) behavior. If, when heated, a polymer solution separates into two immiscible liquid phases, the polymer/solvent system is said to display LCST behavior, and the uncross-linked polymers are described as temperature-sensitive or temperature-responsive. LCST behavior of many aqueous polymer systems has been recognized for over three decades. In 1968, Heskins and Guillet [31] first reported LCST behavior for aqueous solutions of poly-*N*-isopropylacrylamide (NIPA). In 1975, Taylor and Cerankowski [32] described
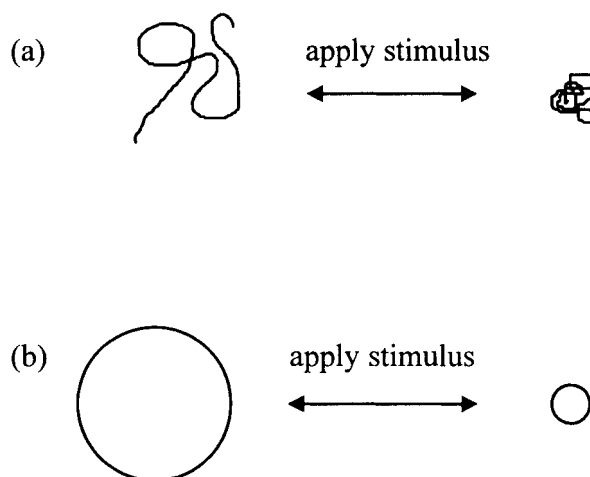

Figure 1. Relationship between a transition in dimension of a single, environmentally responsive polymer chain and a transition in a gel microsphere composed of cross-linked, environmentally responsive polymers. (a) In response to a stimulus such as a change in temperature or pH, an individual, environmentally responsive polymer chain undergoes a reversible transition from an extended to a collapsed state. (b) In response to the same stimulus applied in Fig. 1a, an environmentally responsive gel microsphere undergoes a reversible transition from a large volume to a small volume. Cross-linked environmentally responsive polymers form the gel microsphere. The applied stimulus causes the individual chains in the microsphere to collapse, and the microsphere shrinks as a result. By reversing the stimulus, the microsphere returns to its original volume.

numerous polymers that exhibit LCST behavior in aqueous solutions and demonstrated changes in swelling of corresponding polymer films at the LCST. In 1981, Hrouz *et al.* [33, 34] reported that cross-linked gels of poly(*N*, *N*'-diethylacrylamide; DEA) collapse in aqueous solution at the same temperature at which aqueous solutions of uncross-linked poly(DEA) phase separate. Interest in temperature-induced gel collapse has grown ever since.

Figure 1 illustrates the general analogy between gel volume collapse and collapse of individual, uncross-linked polymer chains. In Fig. 1a, a stimulus causes an individual polymer chain to undergo a coil-globule type of collapse transition. In a polymer solution where all chains collapse simultaneously, liquid-liquid phase separation may be observed, depending on polymer concentration. In Fig. 1b, a gel has been formed by cross-linking many responsive (sensitive) polymer chains shown in Fig. 1a. Upon applying the same stimulus, the individual chains of the network collapse, causing the entire network to shrink as well. In a cross-linked network (gel), the situation is more complex than in a dilute solution of individual, noninteracting chains because the presence of cross-links and entanglements constrains the action of each chain. However, the same, fundamental thermodynamic phenomena drive the transitions in Figs. 1a and 1b. A well-known example of a temperature-sensitive gel is the poly-NIPA hydrogel, the volume of which expands several hundred percent as temperature is lowered from 34°C to 32°C [35]. The volume transition in a cross-linked poly-NIPA gel is driven by a thermodynamic phase transition [35] analogous to the temperature-driven separation of a solution

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* By prepolymerized, we mean that the user does not need to polymerize monomers to cast the separation media, but instead uses uncross-linked, soluble polymers to prepare the gel.
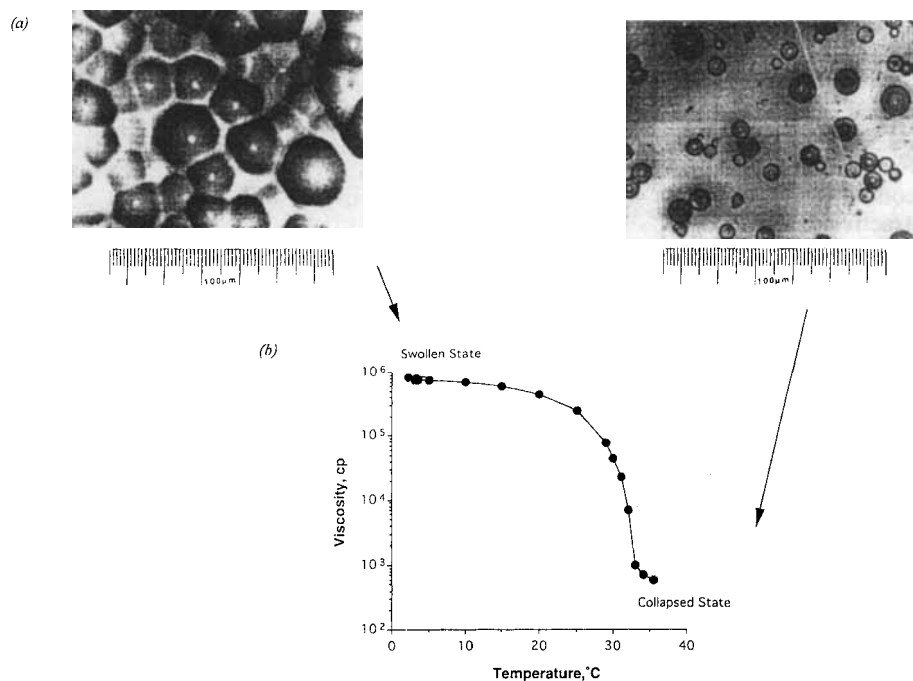
Figure 2. Viscosity transition for a suspension of poly-NIPA gel microspheres. (a) Photographs of a microgel suspension at temperatures immediately below and above the phase transition. Above the transition temperature (35 °C and higher), the microspheres are collapsed, and the suspension has a low viscosity suitable for pouring or loading into narrow channels. Below the transition temperature (30 °C and lower), the microgels expand to form a sieving matrix. (b) Temperature-dependence of viscosity. In this case, the viscosity declines by over three orders of magnitude as a result of the phase transition behavior of the microspheres.

of linear poly-NIPA into two coexisting liquid phases, one rich in polymer and the other rich in water [31]. Other gels in the general *N*-alkylacrylamide family also display temperature-driven volume transitions [36].

1.3.2 Viscosity transitions in temperature-sensitive polymer systems

Previous studies of environmentally responsive gels have focused on the volume changes of individual gel elements [24, 25, 37–39]. The thermodynamics, kinetics, physicochemical properties and applications of responsive gels have been discussed recently in Volumes 109 and 110 of *Advances in Polymer Science*. In this work, we are not concerned directly with volume transitions of monolithic gels; this phenomena by itself has little utility in electrophoresis and is typically avoided. Instead, we have investigated systems of LCST polymers where the phase transition behavior is used to drive a viscosity or sol-gel transition.

Here we demonstrate that, under certain conditions, the phase transition of individual polymeric elements (microspheres or uncross-linked chains) induces a dramatic change in a bulk property, the viscosity, of a suspension or solution containing many such elements [40]. Under certain conditions, we have observed reversible viscosity transitions without bulk phase separation or aggregation in suspensions and solutions containing temperature-sensitive polymers. This viscosity responsiveness opens a new realm of potential applications. In this study, we report the feasibility of novel, thermoreversible polymer formulations for electrophoresis of DNA fragments. Here, we use the viscosity transition so that polymeric media for electrophoresis can be poured or loaded more easily. In addition, the ability to control chain dimensions with temperature enables active control of sieving properties during a run, a possibility which we have investigated in capillary electrophoresis.

These viscosity-responsive (LCST) polymer systems investigated here fall into two classes: (i) suspensions of environmentally sensitive gel microspheres and (ii) solutions containing temperature-sensitive uncross-linked polymers. Both types of temperature-sensitive formulations may exhibit a reversible change of viscosity of several orders of magnitude. We can take advantage of the viscosity change to deliver (*e.g.* pump or pour) a prepolymerized medium at low viscosity into a cavity and subsequently convert the medium into its high viscosity, non-flowing state. Prepolymerized, thermo-reversible media are less toxic because monomers are absent, and these media are more convenient to use than media which the user must polymerize.

Figure 2a is a photograph illustrating the basis for a viscosity, or sol-gel, transition in a suspension of poly-*N*-isopropylacrylamide gel microspheres. Below the transition temperature of an individual hydrogel, each microsphere is swollen. If the volume fraction of expanded microspheres is high, a suspension is extremely viscous, and, in some cases, solid-like. Above the transition temperature, each microsphere is collapsed. If the volume fraction of collapsed microspheres is small, a suspension is highly fluid. Figure 2b illustrates a viscosity transition of approximately three orders of magnitude for a suspension of poly-NIPA microspheres in deionized, distilled water (8.9 % dry solids content). Thus, under appropriate conditions (controlled swelling ratios in expanded and collapsed states, and a corresponding percentage of solids), expansion or collapse of individual gel microspheres causes a sol-gel or gel-sol transition, respectively.

Composite matrices containing temperature-sensitive, uncross-linked polymers behave in a similar manner. When solutions containing temperature-sensitive uncross-linked polymers are heated, the individual polymer chains collapse, and the viscosity of the bulk solution decreases dramatically. Upon cooling, the solution

regains its initial high viscosity. If a rigid gel is required, an appropriate binding or gelling agent can be added, and a warm solution will set to become a solid gel. Other researchers have studied composite matrices for slab gel separations of nucleic acids or proteins. Previous work has examined two types of matrices: composites of cross-linked polyacrylamide and agarose [41–50] and composites of uncross-linked polymers and agarose [41, 51–55]. Most work has involved composite matrices of agarose and cross-linked polyacrylamide. Here the agarose improves the handling properties of polyacrylamide when it is polymerized at extremely low %T. Chiari *et al.* [56] investigated composite gels containing allyl-modified agarose, underivatized agarose, and polyacrylamide, wherein the acrylamide is cross-linked by the activated agarose. These composites provide excellent separation and mechanical properties but still require polymerization of acrylamide monomer in the presence of the activated and unmodified agaroses. Composite matrices of agarose and uncross-linked polymers have been studied by Bode [41]. A limitation is that fine resolution requires high concentrations of uncross-linked polymer, rendering these composites too viscous to pour. In previous studies of composite gels containing uncross-linked polymers, the uncross-linked polymers were not temperature-sensitive. If responsive polymers are used in composite matrices with agarose, the pouring viscosity can be reduced greatly because of the large transition which occurs upon heating. Thus, we can pour gels at polymer concentrations that would ordinarily be too viscous. Furthermore, if we use gel microspheres, we decouple network formation and gelation. Network microstructure is fixed during polymerization of the microspheres, and sol-gel transitions are achieved through reversible volume transitions, rather than through attractive forces between polymer chains.

2 Materials and methods

2.1 Preparation of gel microspheres

Gel microspheres were prepared by suspension or precipitation polymerization. Poly-*N*-isopropylacrylamide gel microspheres were prepared by precipitation polymerization in a one-liter reaction vessel with overhead stirring by the following recipe: 9.8 g *N*-isopropylacrylamide (Kodak), 0.2 g *N,N'*-methylenebisacrylamide (cross-linking agent) (Sigma), 0.48 g IGEPAL 887 surfactant (Rhône-Poulenc), and 0.195 g IGEPAL 630 surfactant (Rhône-Poulenc) were dissolved in 380 g of deionized, distilled water. We heated the solution to 70 °C and added 20 g of water containing 0.4 g of an initiator, potassium persulfate (Sigma). The reaction proceeded overnight. The microspheres were recovered by centifugation and found by microscopy to be less than one micron in diameter. We measured the polymer content of a suspension by drying to constant mass in an oven at 100 °C, reported as %p. As used here, %p relates only to the polymer solids content of the suspension and is obtained by drying a sample of suspension (with no buffer salts present) to constant weight: mass of dried polymer suspension/mass of wet polymer suspension × 100. Microgels of other *N*-substituted acrylamide com-

positions were prepared and recovered by suspension polymerization. First, we prepared an aqueous solution containing monomers, cross-linker, and initiator. For example, to prepare poly-*N,N'*-dimethylacrylamide (DMA)/DEA microspheres, we dissolved 6 g DMA (Polysciences), 6 g DEA (Monomer Polymer Dajac), 0.12 g *N,N'*-methylenebisacrylamide (JT Baker), and 0.16 g ammonium persulfate (Sigma) in 87.88 g deionized, distilled water. Next we suspended the aqueous solution in an organic solvent by overhead, mechanical stirring in a closed reaction vessel. For the poly-DMA/DEA microspheres, we used as the organic solvent 350 mL 1,1,1-trichloroethane (Aldrich) in which 0.175 g ethylcellulose (Aldrich; 4 cp at 5% solution) had been dissolved. The suspension was sparged continuously with nitrogen. To initiate the free-radical reaction, an accelerator, 1 mL TEMED (Sigma), was added to the suspension. Reaction progress was monitored via temperature of the suspension; in all cases we allowed for an excess of time to insure complete reaction. For the poly-DMA/DEA microspheres, the maximum temperature rise was 4 °C. Microspheres were recovered as an aqueous suspension by repeated washing and centrifugation steps using water and an organic solvent such as acetone. As desired, suspensions were further concentrated by rotary evaporation under heating or by freeze-drying.

2.2 Preparation of uncross-linked polymers for capillary and slab gel electrophoresis

Uncross-linked polymers were prepared by free-radical solution polymerization. Polymerization was performed following methods standard in the capillary electrophoresis field for polymerization of uncross-linked polyacrylamide. For example, for sequencing experiments by CE, we dissolved 7.2 g of DMA (Polysciences) and 16.8 g of DEA (Monomer-Polymer Dajac) in 376 mL deionized, distilled water in a Pyrex bottle and sparged the solution with helium for 7 h. To initiate the reaction, 752 µL of 10% w/v ammonium persulfate (Sigma) solution and 376 µL of TEMED (Sigma) were added rapidly. The bottle was sealed, and the solution was stirred slowly at room temperature for 30 min. The bottle was then transferred to a refrigerator at 4 °C. The reaction was allowed to proceed for 24 h. For slab gel electrophoresis, we prepared temperature-sensitive polymers in exactly the same manner, except that we sparged with helium for only 1 h, and we used the following recipe: 12 g DMA and 12 g DEA in 376 mL deionized, distilled water with 320 µL of 20% w/v ammonium persulfate solution and 320 µL of TEMED. After polymerization, the viscous solution was transferred to a freeze-drying flask and placed in a freezer for 48 h. The frozen polymer solution was lyophilized for three days using a LabConco Freeze One. The resulting dried polymer was then redissolved in buffer.

2.3 Preparation of electrophoresis matrices

Microgel suspensions were prepared for electrophoresis by mixing recovered microgels with cold 0.5 × TBE (44.5 mM Tris, 44.5 mM boric acid, 0.5 mM EDTA) buffer, heating the suspension to above the transition temperature in a water bath, and then loading the suspension
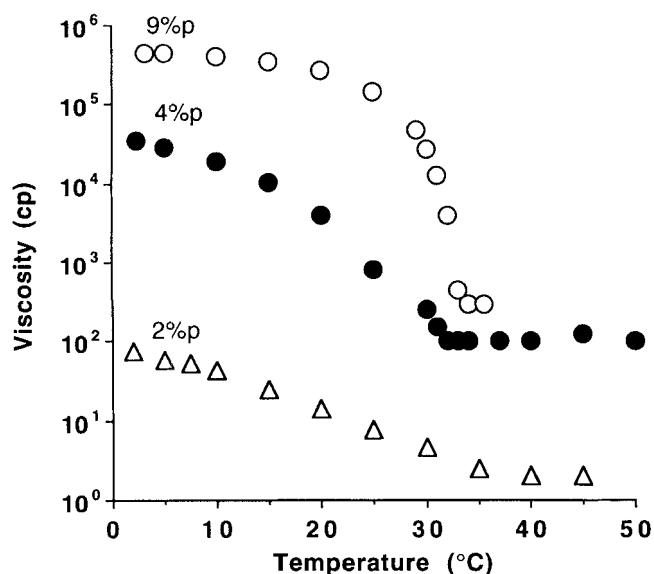
Figure 3. Effect of temperature and %p on viscosity of aqueous suspensions of temperature-sensitive, poly-*N*-isopropylacrylamide microspheres. Increasing temperature causes a sharp and dramatic decrease in the viscosity of a 9%p suspension. As the suspension is diluted (lowering %p), the viscosity transition occurs over a wider range of temperature.

into a UV-transparent tube by means of a disposable syringe outfitted with a piece of Tygon tubing as an adapter. Ethidium bromide (Bio-Rad) was added to a final concentration of 0.5 µg/mL immediately before loading. Matrices containing uncross-linked polymers were prepared for slab electrophoresis by first dissolving dried polymers in 0.5 × TBE buffer at 10 °C by tumbling overnight on a laboratory rotator. The desired amount of powdered agarose was then added to the polymer solution. The suspension was heated in a water bath to 95 °C, cooled to 60 °C, and poured into a 15 × 20 cm casting tray for the Hoefer Max Submarine Electrophoresis Unit (HE99X). Ethidium bromide was added to a final concentration of 0.5 µg/mL immediately before loading. A 1.0 mm comb was inserted, and the gel was allowed to set at room temperature. Matrices containing uncross-linked polymers were prepared for capillary electrophoresis by dissolving polymers in the desired buffer. Solutions were shaken vigorously by hand and mixed overnight on a laboratory rotator. Capillaries were coated with polyacrylamide following the procedure of Hjertén [57]. The 57.5 cm long, 75 µm ID capillary was pressure-loaded externally with solution and run using a Beckman P/ACE System 2100. The external pressure loading was accomplished using a specially designed cell. To load a capillary, we filled the cell partially with polymer solution and threaded a capillary through a pierced ferrule in the top of the cell until the end of the capillary was submerged in solution. The entire cell could be immersed in a controlled-temperature bath. The capillary was filled by pressurizing the cell with nitrogen.

2.4 Measurement of solution viscosity

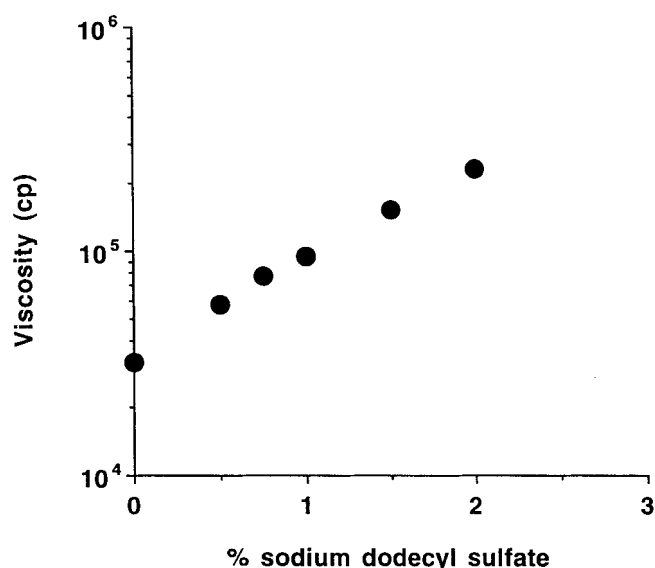To determine the dependence of viscosity on temperature for a solution of uncross-linked polymers, we used a

Figure 4. Effect of surfactant concentration on the viscosity of a suspension of expanded poly-*N*-isopropylacrylamide microspheres at 20.7 °C. Addition of sodium dodecyl sulfate increases viscosity.

Brookfield Model DVII+ viscometer. The viscometer is equipped with a water-jacketed cell; a Brookfield TC500 constant-temperature bath was used to circulate cooling or heating water through the jacket. Viscosity was not recorded until the viscosity reading had stabilized at the desired temperature.

3 Results

In the following sections, we describe the viscosity behavior of temperature-sensitive microsphere suspensions and provide examples of the use of temperature-sensitive polymers in capillary and slab electrophoresis of DNA.

3.1 General characteristics of temperature-sensitive microgel suspensions

We have prepared aqueous suspensions of poly-*N*-isopropylacrylamide gel microspheres and measured the suspension viscosity as a function of temperature and polymer concentration (%p). At temperatures below the transition temperature of an individual hydrogel, each microsphere is swollen. If the volume fraction of expanded microspheres is high, a suspension is extremely viscous and, in some cases, solid-like. At temperatures above the transition temperature, each microsphere is collapsed. If the volume fraction of collapsed microspheres is small, a suspension is highly fluid. Thus, under appropriate conditions (controlled swelling ratios in expanded and collapsed states, and a corresponding percentage of solids), expansion and collapse of individual gel microspheres causes a sol-gel transition. Figure 3 shows the effect of temperature on the viscosity of aqueous poly *N*-isopropylacrylamide microgel suspensions at different %p. The viscosity was measured at a shear rate of 0.1 s^{-1} using a Brookfield DVII+ viscometer with a water-jacketed temperature cell. For the 9%p
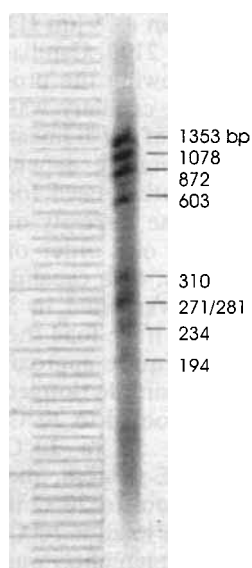
Figure 5. Separation of ΦX174/HaeIII fragments in a suspension of microgels by tube gel electrophoresis. Electrophoresis was performed for 2 h at 7.8 V/cm in 0.5 × TBE buffer. The image has been inverted for contrast. Visible are bands for the 1353, 1078, 872, 603, 310, 271/281, 234, and 194 bp fragments.

suspension, the viscosity falls between 30 and 34 °C from over 100 000 centipoise (cp) to less than 1000 cp. As we dilute the suspension (lowering %p), the transition in viscosity becomes less sharp and occurs over a wider range of temperature. Upon heating, the suspension viscosity decreases because each microsphere collapses, expelling water. The extent of water expulsion determines the magnitude and broadness of the viscosity transition. Thus, we can control the viscosity transition through %p and/or microsphere chemistry and synthesis conditions.

We can also affect the viscosity transition of a suspension of microspheres if we add components which change the swelling transition of each microsphere. Figure 4 shows the viscosity as a function of concentration of a surfactant, sodium dodecyl sulfate (SDS), for a suspension of poly-N-isopropylacrylamide microspheres (8%p) at 20.7 °C (i.e., in the expanded or swollen state). Viscosity was measured at a shear rate of 0.1 s⁻¹. The addition of 2% SDS increases viscosity by more than one order of magnitude. Inomata et al. [58] have reported previously the expansion of poly-N-isopropylacrylamide gel rods in solutions containing SDS.

The examples above demonstrate the universality of sol-gel transitions in microsphere suspensions (above a certain %p) induced by changes in microsphere volume. Suspensions of environmentally responsive hydrogel microspheres represent a new class of "smart" materials. Using the same stimuli that induce gel volume transitions, we can convert these suspensions from a high-viscosity, near-solid gel to a low-viscosity fluid. Like gel volume transitions, viscosity transitions are reversible. Matsuo et al. [37] have studied the kinetics of volume transition for gel microspheres. These studies indicate that rapid viscosity transitions can be obtained, limited

primarily by heat transfer to and from the suspension. Thermoreversible microsphere suspensions can be compared to thermoreversible polysaccharide or polypeptide gels such as agarose, gelatin, and β-1,3 glucans. However, the intrinsic network topology of a microsphere is never broken during cycling of temperature, and gel microsphere suspensions do not exhibit hysteresis.

3.2 Tube gel electrophoresis of dsDNA using gel microspheres

Figure 5 shows separation of ΦX174/HaeIII fragments separated by temperature-sensitive, poly-DMA/DEA (12%T, 1%C) microspheres in tube electrophoresis. After loading the microgel suspension into the 15 cm long, 3 mm ID tubes, we capped the tubes with circles of buffer-moistened filter paper and allowed the tubes to cool; the suspension formed a solid-like pseudocontinuum. Upon setting, the microgel suspension is clear and transparent, similar to a continuous, cross-linked polyacrylamide gel. Electrophoresis was run for 2 h at 7.8 V/cm in 0.5 × TBE in a Hoefer SE 600 tube gel apparatus. The 194, 234, 271/281, 310, 603, 872, 1078, and 1353 bp bands were resolved.

3.3 Capillary electrophoresis of dsDNA using temperature-sensitive polymer solutions

In CE it is desirable to use a replaceable separation medium. Furthermore, because detection is performed on-line, and because thermal and gravitational convection are minimized in a capillary, the separation matrix need not be rigid (self-supporting). For these reasons, the use of uncross-linked polymer has become widespread for CE. Examples of uncross-linked polymers used for capillary electrophoresis include polyacrylamide [7, 8], polyethylene oxide [59, 60], polyethylene glycol [13], and hydroxyethylcellulose [15, 16, 61, 62]. Most researchers have employed polymer solutions of high concentration or moderate concentation and high molecular weight to achieve superior resolution of DNA. However, it is more correct to say that high resolution, i.e., single-base pair resolution, is obtained using solutions of polymers that are highly entangled [63–65]. As a consequence of chain entanglement, polymer solutions providing high resolution are often highly viscous. Viscous polymer solutions often require high pressures and long times to load in narrow-bore capillaries. More than one author has reported that these highly entangled solutions are difficult or impractical to use because loading a 50—1200 μm ID capillary requires a long loading time [64, 66–68]. Solutions of temperature-responsive polymers enable us to load at low viscosity (low entanglement) and run electrophoresis at high viscosity (high entanglement), thereby circumventing the trade-off between viscosity and fine resolution.

To test the feasibility of using a temperature-induced viscosity transition to reduce capillary loading time, we performed capillary electrophoresis of double-stranded DNA using a solution containing two uncross-linked polymers already reported in the CE literature, hydroxyethylcellulose (HEC) and hydroxypropylcellulose
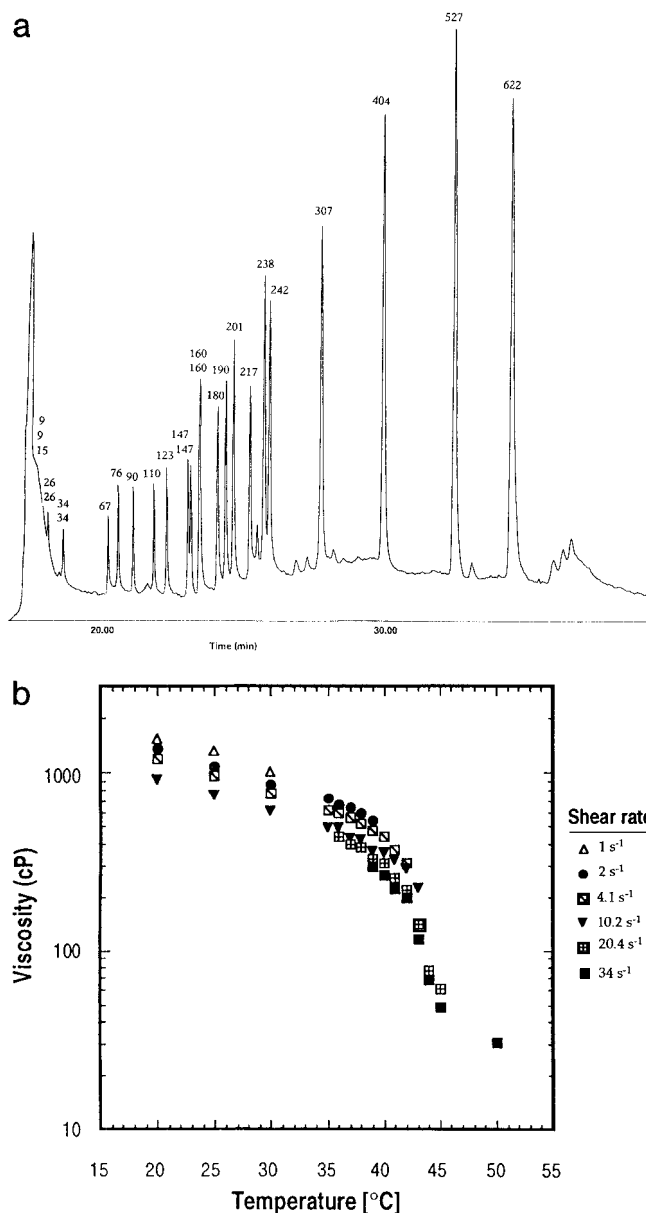
a

b

Figure 6. Use of a temperature-sensitive polymer solution for capillary electrophoresis of double-stranded DNA fragments. (a) Electrophorogram illustrating separation of pBR322/*Msp*I DNA fragments by capillary electrophoresis in a temperature-sensitive polymer solution containing 1.5% HPC and 0.4% HEC. Electrophoresis was performed in 0.5 × TBE buffer at 245 V/cm in a polyacrylamide-coated, 75 µm ID capillary. (b) Dependence of viscosity on temperature for the HPC/HEC solution that provided the results shown in Fig. 6a. The viscosity transition is centered around approximately 42°C.

(HPC) [12]. Solutions of HPC display a viscosity transition when heated, although LCST-type phase separation, as described in Section 1.3.1, was not observed. In Section 3.5, we report results of DNA sequencing by CE using our temperature-sensitive, LCST-type poly-DMA/DEA.

Figure 6a illustrates the separation of pBR322/*Msp*I DNA fragments by CE in a temperature-sensitive solution containing 1.5% HPC (SP^2; M_r 60000) and 0.4% HEC (Polysciences; M_r 90000–105000). Electrophoresis

was performed at 245 V/cm in 0.5 × TBE in a 75 µm ID capillary using a Beckman P/ACE System 2100. Capillaries were coated with polyacrylamide following the procedure of Hjertén [57]. The 57.5 cm long capillary was pressure-loaded externally with a solution of 1.9% linear polymers. Figure 6b illustrates the temperature dependence of viscosity of the HEC/HPC solution used for Fig. 6a. When heated from 20 to 50°C, the viscosity of the HEC/HPC solution declines by over one order of magnitude. At a loading pressure of 20 psi (the capability of the P/ACE System), the capillary was filled in 3 min at 50°C using an off-line, loading cell. If we load the capillary at 20°C and 20 psi instead, we require approximately 33 min, over one order of magnitude longer. Before starting the run, the capillary was cooled to 20°C. Although cooling of the capillary from 50 to 20°C requires several minutes with the P/ACE system, the total time required to load at 50°C and cool to 20°C is significantly less than the time required to load at 20°C. Excellent size-based resolution of all fragments was obtained, including separation of the 238 bp and 242 bp fragments.

3.4 Slab gel electrophoresis of dsDNA using thermoreversible polymeric media

Slab gels are by far the most widely used electrophoresis format. Here, not as in CE, the electrophoretic matrix must support itself, *i.e.*, it must be firm or rigid to endure handling and transfer from one container or surface to another. Any matrix used for conventional slab gel electrophoresis must include a gelling, binding, or cross-linking agent, particularly when used for horizontal submarine electrophoresis. For example, polyacrylamide matrices are rigidified by chemical reaction of acrylamide with a cross-linking agent, and agarose matrices are inherently rigid because agarose is a gelling agent. As described in Section 3.1, heated suspensions of microgels become gel-like upon cooling. We have performed electrophoresis in aqueous suspensions containing microgels; however, the resulting slabs are often weak and difficult to pick up. By incorporating a small amount of gelling material, we have developed matrices which can be easily handled during common, postelectrophoretic activities such as staining, electroblotting, and transillumination.

Figure 7a illustrates separation of DNA fragments in a slab gel formulation based on temperature-sensitive polymers. Electrophoresis was run for 2 h at 6.4 V/cm in 0.5 × TBE in horizontal format. We prepared the gel by the heat-and-pour method, adding 0.7% agarose to a pre-dissolved solution of 0.75% HEC and 0.75% poly-DMA/DEA. The uncross-linked polymers were dissolved prepared by tumbling a solution overnight. From left to right, the DNA fragments are: 10 bp ladder, 100 bp ladder, and ΦX174/*Hae*III. Resolution of a 10 bp ladder up to approximetely 150 bp was achieved, and all bands were resolved in the ΦX174/*Hae*III and 100 bp ladder. Separation of the 271 and 281 bp fragments of ΦX174/*Hae*III demonstrates the feasibility of this matrix to separate small fragments only 4% different in length. Similar resolution can be obtained from composite matrices containing agarose and concentrations of HEC
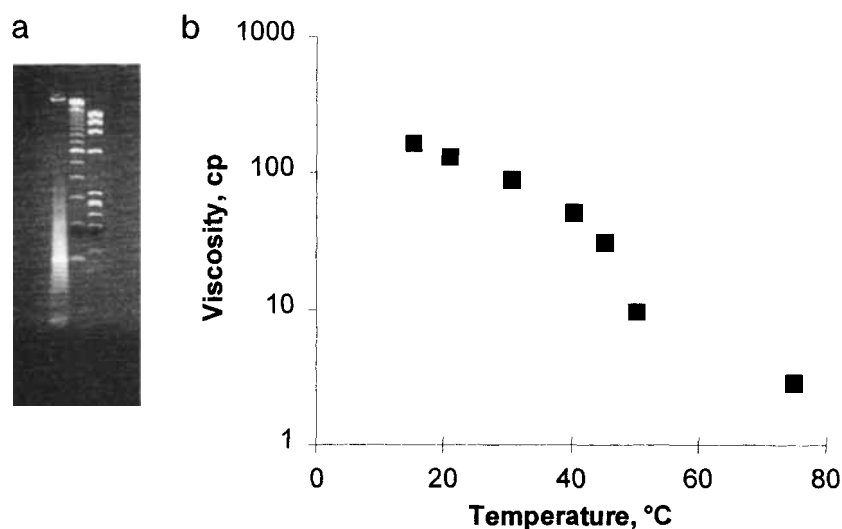
Figure 7. Use of a composite gel containing uncross-linked, temperature-sensitive polymers for submarine electrophoresis of DNA. (a) Separation of dsDNA fragments from 10 to 1500 bp in a horizontal composite slab gel containing temperature-sensitive polymer. DNA samples are: lane (1) 10 bp ladder; (2) 100 bp ladder; (3) ΦX174/*Hae*III. Electrophoresis was performed for 2 h in 0.5 × TBE buffer at 10 V/cm. Separation of the 10 bp ladder and 100 bp ladder occur simultaneously, as in cross-linked polyacrylamide. The 10 bp ladder is resolved to approximately 150 bases, and the 271/281 bp fragments of ΦX174/*Hae*III are resolved (separation more visible on original gel). (b) Dependence of viscosity on temperature for a 2% solution of poly-DMA/DEA used in the composite gel illustrated in Fig. 7a. The polymer was dissolved by tumbling overnight in 0.5 × TBE buffer. The viscosity transition is gradual and occurs between 40 and 80°C.
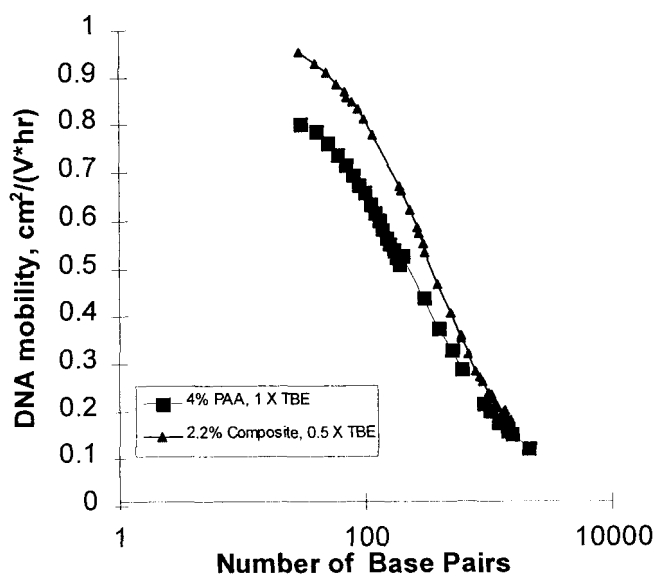

Figure 8. Mobility of dsDNA fragments during electrophoresis in cross-linked polyacrylamide and the matrix shown in Fig. 7a. Samples of the 10 bp ladder and of the 100 bp ladder were run on a vertical, cross-linked 4% polyacrylamide gel in 1 × TBE; these samples were also run on a horizontal, 2.2% Soane BioSciences matrix in 0.5 × TBE. Fragment mobilities are similar in our matrix and in polyacrylamide.

greater than 0.75%; however, these composite gels are difficult to pour because of their viscosity, as reported previously [52]. Figure 7b illustrates the temperature-dependence of viscosity for the poly-DMA/DEA used in the composite slab shown in Fig. 7a. For this particular poly-DMA/DEA composition, the viscosity transition is gradual and occurs between approximately 40–80°C.

Figure 8 shows a comparison of fragment mobilities in 4% cross-linked polyacrylamide and the gel illustrated in Fig. 7a. The cross-linked polyacrylamide gel prepared by following the procedure of Sambrook *et al.* [69] using an acrylamide:*N*,*N'*-methylenebisacrylamide ratio of 29:1 by weight. Electrophoresis of a 10 bp and 100 bp ladder was performed in a Hoefer SE 600 Vertical Unit at 8 V/cm

for 2 h in 1 × TBE. The mobility of small fragments in the composite slab gel was slightly faster than in the cross-linked polyacrylamide gel.

3.5 Temperature-sensitive uncross-linked polymers as replaceable media for DNA sequencing by CE

We have also investigated the applicability of temperature-sensitive media for separation of DNA sequencing products by CE. DNA sequencing is especially demanding because single-base resolution is essential, generally requiring highly entangled and viscous solutions. Figure 9 shows separation of sequencing fragments by capillary electrophoresis using a solution of 6% temperature-sensitive polymer (poly-DMA/DEA of composition described in Section 2.2) in 1 × TBE buffer with 7 M urea. Solutions of this polymer underwent a gradual viscosity transition as the solution was heated from approximately 55 to 70°C. Oligonucleotides were generated by T-terminated primer extension of an M13mp18 template using a Sequenase Sequencing Kit (Amersham Life Science). Oligonucleotides were injected for 15 s at 12 kV, and electrophoresis was performed in a 70 cm (45 cm effective) polyacrylamide-coated capillary at 12 kV and ambient temperature. Single-base resolution was observed up to approximately 150 bases. The viscosity of this matrix was extremely low; at 70°C, above the transition temperature, the viscosity of this matrix is 10 cP. These data should be regarded as a demonstration of feasibility of single-base separation by this type of temperature-sensitive media; further optimization is ongoing to improve resolution and extend read length.

4 Discussion

We have shown that temperature-sensitive polymers are a versatile media for applications in electrophoresis of DNA. They can be used in several common formats (slab, tube, capillary) and are compatible with existing protocols and methods in electrophoresis. The two classes of formulations we have investigated, gel microsphere suspensions and solutions of uncross-linked polymers, have an important common feature because of their temperature responsiveness: low pouring or loading
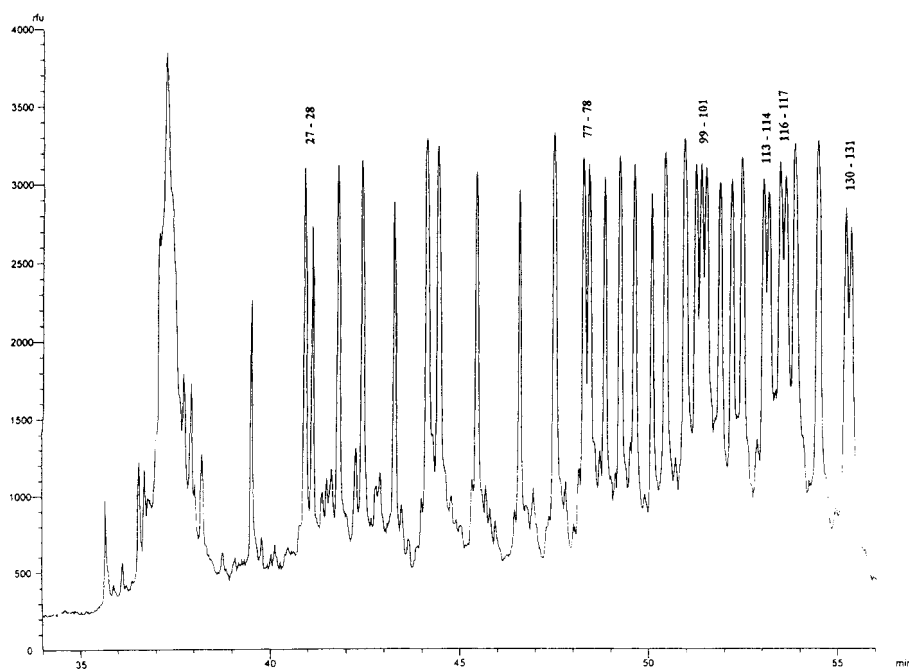
Figure 9. Separation of oligonucleotides by CE in a temperature-sensitive matrix containing 6% uncross-linked poly-DMA/DEA, 7 M urea and 1 × TBE. Single-base resolution was achieved.

viscosity. In addition, each class of formulation has distinguishing features. Compared to uncross-linked polymer solutions, microgel suspensions have a large interfacial surface area by virtue of the particulate nature of the system. Therefore, surface properties are an important determinant for electrophoretic separation. We have resolved DNA fragments in capillary, tube, and slab formats, and in each case, the final packing of the microspheres is an important determinant of the resolving ability. The two classes of formulations exhibit equivalent resolving power over different ranges of polymer concentration. For example, to separate the fragments of the ΦX174/*Hae*III digest, we require more than twice the polymer concentration (on a dry basis) if we use a microgel suspension than if we use a solution of uncross-linked polymers. The examples here demonstrate the feasibility of using temperature-sensitive polymer systems in electrophoresis of DNA. In addition, the ease-of-use, nontoxic nature, and potentially high resolving power make the development of such media a worthwhile endeavor.

5 References

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