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Nanostructured Copolymer Gels for dsDNA Separation by Capillary Electrophoresis

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Abstract

Pluronics copolymers are triblock copolymers of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) and are able to form many different ordered nanostructures at appropriate polymer concentrations and temperatures in selective solvents. These nano-structured ‘gels’ showed desirable criteria when used as DNA separation media, especially in microchip electrophoresis, including dynamic coating ability and viscosity switchable property. A ternary system of F127 (E₉₉P₆₉E₉₉)/TBE buffer/1-butanol was selected as a model system to test the sieving performance of different nanostructures in separating dsDNA by capillary electrophoresis. The lattice structures were determined by small-angle x-ray scattering with quasi-lattice crystal parameters being calculated according to the x-ray scattering data. Viscosity measurements showed the sol-gel transition phenomena. In addition to the cubic structure, successful electrophoretic separation of dsDNA in 2-D hexagonal packed cylinders was achieved. Results showed that without further optimization, ΦX174 DNA-Hae III digest was well separated within 15 minutes in a 7-cm separation channel, by using F127/TBE/1-butanol gel with a 2-D hexagonal structure. A mechanism for DNA separations by those gels with both hydrophilic and hydrophobic domains is discussed.

Keywords

2-D hexagonal; DNA separation; Nanostructures; Pluronics

1. Introduction

Triblock copolymers of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) or E_xP_yE_x, with E, P, and subscripts denoting oxyethylene, oxypropylene, and segment length, respectively, have been studied extensively in many fields, such as detergency [1–3], emulsification [4–6], and drug delivery [7–11]. Many such triblock copolymers are commercially available and known as Pluronics (BASF) or Poloxamers (ICI). An unusual feature of these triblocks is that they can self-assemble into quasi-crystalline lattice nanostructures in selective solvents. In aqueous solution, the copolymer often exists as unimers and exhibits a low solution viscosity even when the copolymer solution concentration is fairly high because E and P blocks are both soluble in water at low temperatures and the copolymer molecular weight is relatively low [12–14]. When the temperature goes up beyond the sol-gel transition temperature at an appropriate polymer concentration, the polymer chains will aggregate into micelles with a dense core formed by PPO blocks and a shell composed of hydrated PEO chains mainly due to a breakdown of hydrogen bonds between PPO and water molecules. At high enough concentrations, the micelles come in contact and overlap with one another, leading to a sharp increase in

viscosity. Finally, a gel-like state will occur with ordered quasi-lattice structures being formed. Another noticeable property of these copolymers is that they can be tuned to the desired morphology over a range of morphology with different length scales, since the chain length of E or P can be controlled by the polymerization process [15–17]. Both experimental data and theoretical predictions have shown that the self-assembled structures can be selectively achieved by adjusting the polymer molecular weight, the ratio of E/P, the solvent type, and the copolymer/solvent composition [18–20].

The formation of nanostructures has drawn increasing attention to its possible application in the DNA separation field by capillary electrophoresis due to their unique viscosity-adjustable properties, plus the dynamical coating ability of PEO [21,22]. They can be injected into the capillary (typical inner diameter of less than 100 μm) at relatively low pressures and at low (or room) temperatures. The separation of DNA will be performed when the polymer solution is in its gel-like state. For example, the matrices, composed of ($\text{E}_{99}\text{P}_{69}\text{E}_{99}$), have succeeded in separating double-stranded DNA [22–27] and oligonucleotides [21,23,28,29]. The “viscosity switch” property is particularly desirable when applied on a micro-fluidic chip, the promising technique has the *potential* to increase the sequencing rate by 5–10 fold to obtain a similar read length as that in the current capillary array electrophoresis (CAE) technology [30–32].

Oligonucleotide sizing markers ranging from 8 to 32 base could be successfully separated in a 1.5 cm long separation channel within 40 s on a microchip electrophoresis instrument (Agilent Bioanalyzer 2100) [21], using a mixture of F127 and F87 (2:1, weight ratio) with an overall concentration of 30 wt%. Body-centered cubic (BCC) structures formed by $\text{E}_{45}\text{B}_{14}\text{E}_{45}$ (B20-5000, with B denoting poly(oxy butylene) copolymer could also separate the same oligonucleotides in the same separation length [21]. Another example is that a mixture of two triblock copolymers ($\text{B}_6\text{E}_{46}\text{B}_6$ and $\text{B}_{10}\text{E}_{271}\text{B}_{10}$) was tested as a sieving matrix for dsDNA fragments by capillary electrophoresis, although still with an unresolved ordered structure [33]. Furthermore, it has been demonstrated that with a cubic structure of Pluronics F127, capillary gel electrophoresis could separate DNA molecules ranging over several categories from single-stranded DNA to double-stranded DNA, including nucleoside monophosphates and organic dyes, oligonucleotides of 4–60 nucleotides, DNA fragments of 50–3000 base pairs, to super-coiled plasmid DNA of 2000–10,000 base pairs [34–37]. Thus, the usage of nanostructured gels of Pluronics has opened a new window for DNA separation over a wide range of sizes and will contribute to the process of lab-on-a-chip. It is noted that the mechanism of DNA sieving in quasi-lattices should be different from other random polymer gels, as there are more discrete and periodic hydrophobic and hydrophilic domains. However, a definitive mechanism, as well as a clear relationship between structure and sieving performance, requires further study. It is intriguing to find why the small oligonucleotides and big plasmid dsDNAs had roughly similar motilities in the FCC structures formed by 25% F127 gels. [36]

Interestingly, the ordered structure used in DNA separation has been *only* cubic, including face-centered [22,26,38] and body-centered [21,23,28,29] cubic. No experimental data were reported on DNA separations using ordered nanostructures, such as lamellar or hexagonal structures, other than the cubic packing of spherical micelles. One reason could be that Pluronics triblock copolymers are assembled into “non-cubic” structures only at very high polymer concentrations in the normal operating CE temperature range in aqueous solution.

Using Pluronics F104 ($\text{PEO}_{18}\text{PPO}_{58}\text{PEO}_{18}$) as an example, the copolymer begins with an isotropic solution in dilute solution, followed by micellization, the formation of cubic phase with spherical micelles, a hexagonal phase of columnar micelles, and then a lamellar phase [39]. On the other hand, F127 does not show ordered structures in aqueous solution other

than the cubic structure, with polymer concentrations going up to 70 wt%. This inaccessibility over the temperature and polymer concentration range may be an explanation as to why no experimental data have been reported on the usage of hexagonal or lamellar phases in DNA separation [17,39].

Investigating the sieving behavior of nano-structured polymer gels over a wider range of quasi-lattice structures, in addition to the cubic quasi-lattice structure, should be of interest. Its importance derives not only from a fundamental point of view concerning the mechanism of DNA separation in ordered gel structures and related issues, but also from useful information that may guide us to further matrix modifications for the separation of DNA or other polyelectrolytes.

The introduction of a second solvent (e.g., 1-butanol.) to the system can result in the formation of hexagonal or lamellar structures at a *moderate* polymer concentration [40]. With this information, F127/TBE/butanol was chosen as the model solution, because both hexagonal and lamellar structures can be formed with the concentration of F127 in the range from 20% to 80% and 10% to 35% (wt%), respectively. This span of polymer concentration could cover the current concentration range applied to DNA separation by CE.

Organic solvent has been previously used in the DNA separation medium. It has also been employed in the interest of selectivity for DNA separation [41,42]. For example, Roeraade *et al* used *N*-methylformamide (NMF) containing 50 mM ammonium acetate as a solvent to dissolve C₁₆-derivatized 2-hydroxy-ethyl cellulose (HEC) and succeeded in baseline separation of p(dA)_{12–18} and p(dA)_{40–60} oligonucleotides [43]. A second example is that Karger *et al* applied a mixture of 2 M urea with 5% v/w of dimethylsulfoxide (DMSO) as a denaturant, resulting in a read length of 975 bases at 70 °C in 40 minutes with 98.5% accuracy by using LPA as the sieving matrix [44]. Hence, a small fraction of proper organic solvent may be tolerable in the separation medium. Nevertheless, it is important to acknowledge the fact that more complex systems are often inferior to simpler basic systems, especially in terms of reproducibility of data and quality control.

2. Materials and Methods

2.1 Chemicals

Plurionics F127, PEO₉₉PPO₆₉PEO₉₉ or E₉₉P₆₉E₉₉ was a gift from BASF Corporation (Parsippany, NJ, USA). The DNA standard sample ΦX174 DNA-Hae III digest and the staining dye ethidium bromide were purchased from Sigma Chemical (St. Louis, MO, USA). Tris(hydroxymethyl)aminomethane, boric acid and EDTA were purchased from Aldrich (Milwaukee, WI, USA). 1-butanol was purchased from Fisher Scientific Co. (Springfield, NJ, USA) and used directly.

The water used in all the reactions and solutions was de-ionized to 18.2 MΩ with a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2 Sample preparation

The triblock copolymer solution was prepared by first dissolving in 1×TBE buffer, followed by mixing with 1-butanol to desired fractions. All solutions were vortexed sufficiently to obtain a uniform system and then stored in the refrigerator before use.

The 1×TBE buffer solution (pH = 8.3) was composed of 89 mM boric acid, 89 mM Tris(hydroxymethyl)aminomethane, and 2.0 mM EDTA.

The DNA was diluted from the original concentration of 1,000 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$ with Milli-Q water.

2.3 Viscosity measurements

Viscosity measurements were carried out on a Physica MCR301 (Anton Paar, VA) rheometer. Parallel plates with a 1 mm gap were used to perform the measurements. The solutions were sealed with high-boiling-point silicone oil so as to avoid solvent evaporation. A shear rate of 0.5 s^{-1} was applied. The viscosity versus temperature was taken with a temperature increment of 1°C in the range of 10°C to 50°C .

2.4 Capillary electrophoresis

A lab-built capillary electrophoresis system with laser-induced fluorescence detection was used to perform the DNA separation, as stated in reference [45]. A 10-cm long silica fused capillary, purchased from Polymicro Technologies (Phoenix, AZ, USA), with id/od of 50/365 μm was employed to run the CE experiments. A detection window was opened at 3 cm from the cathode end by stripping off the polyimide coating with a razor blade. The capillary was first flushed with 1 M HCL for 10 minutes and then rinsed for another 10 minutes with Milli-Q water. The separation media were pumped into the pre-treated capillary by a 50- μl Pressure-Lok® Precision Analytical Syringe (VICI Precision Sampling Inc., Baton Rouge, LA, USA) in the solution state at corresponding temperatures. The cathode and anode ends were immersed in the reservoirs filled with $1\times\text{TBE}$ buffer and $1\times\text{TBE}$ buffer plus 3 $\mu\text{g/ml}$ EB dye, respectively. A pre-run at a voltage of 200 V/cm for 5 minutes before each electrophoresis experiment was conducted so as to electro-kinetically introduce the EB dye into the capillary and to stabilize the current. The DNA sample was injected into the capillary at a constant applied voltage of 300 V/cm for 5 seconds. The DNA separation was run at a field strength of 200 V/cm. To replace the separation medium, the gel-like copolymer matrix could be transformed back to the solution state by holding the capillary at an appropriate temperature for 10 minutes. The capillary was then refreshed by driving out the solution using 1 ml Milli-Q water, followed by rinsing with 1 M HCL over a period of 10 minutes before the next introduction of copolymer solution.

2.5 Small-Angle X-ray Scattering (SAXS)

SAXS experiments were performed on the X27C beamline at the National Synchrotron Light Source (NSLS), Brookhaven National Laboratory (BNL) [46]. The detailed experimental setup has been published in reference [47]. The incident beam wavelength was 0.1371 nm. A three-pinhole collimation system was used to define the incident beam from a double multi-layered monochromator. A Fuji HR-V imaging plate ($200\times 250 \text{ mm}^2$) was used to record the static scattering pattern. The sample-to-detector distance was 1857 mm for the imaging plate.

3. Results and Discussions

3.1 Pluronic copolymer nanostructures in binary and ternary systems

To date, F127 or its mixture with a second triblock copolymer (i.e. F87, $(\text{E}_{61}\text{P}_{40}\text{E}_{61})$) has been among the two most widely used triblock copolymers as sieving media for DNA separations. The experiments included both dsDNA [22,26,35,36,38,48–50] and oligonucleotides [21,23,29,34–36]. However, no data has ever been reported on the separation of DNA in lattice structures other than the cubic structure. Thus, one fundamental question could be whether ordered structures, other than those of cubic ones, can be used in sieving DNA separations. One may also question whether other Pluronic copolymers could produce hexagonal or lamellar structures with an appropriate mesh size, or that these

lamellar or hexagonal structures cannot form ordered structures over a large enough length scale for DNA separations. The cubic structure is isotropic, while hexagonal or lamellar structures are anisotropic and involve local domain orientations. It should perhaps also give us a hint on how the separation matrices should perform with respect to micro-structures. Obviously, the different chain length of PEO and PPO/PBO block must be critical for the separation behavior for a particular size range of DNA, as the nano-structures shall determine the mesh sizes of the network in the gel-like state under a given temperature and copolymer concentration. Hence, we chose F127 as our *model* copolymer because it had been well established that F127 had an appropriate chain length and E/P ratio and could effectively separate dsDNA standard sample Φ X174 DNA-Hae III digest.

A much more complex structural polymorphism of Pluronics copolymers has been observed in the ternary system with two solvents. One is good for PEO blocks (i.e. water), while the other is selective for dissolving PPO, such as xylene, butyl acetate or 1-butanol [40]. Among them, 1-butanol was selected because hexagonal and lamellar structures occurred over similar polymer concentrations. In addition, the lattice structures could be formed in water with a fairly low fraction of organic solvent at the desired polymer concentration range (10 ~ 25%, wt%). The selected organic solvent was of low toxicity and relatively inexpensive. SAXS measurements were carried out to determine the structures. The results will be discussed in the next section.

The F127/TBE binary system behaves as a free flow Newtonian fluid at low temperatures. When the temperature is increased to its sol-gel transition temperature, the solution becomes gel-like with a sharp increase in the viscosity at a given copolymer concentration [39]. However, the behavior was different for the ternary system with butan-1-ol as the second solvent. Data showed that the mixture of F127/TBE/1-butanol exhibited a high viscosity at low temperatures. The viscosity decreased gradually along with increasing temperature. Figure 1A is the viscosity versus temperature curve of a mixture of 22% F127, 61% TBE and 17% 1-butanol (wt%). It showed a viscosity of 30,000 cp at 10 °C, and decreased by about two orders of magnitude as the temperature was increased to 50 °C at a fixed shear rate of 0.5 S⁻¹. The change in viscosity with increasing temperature for the sample with the same weight fraction of F127, but with a higher fraction of 1-butanol of 0.28 (weight fraction) exhibited the same tendency (Figure 1B) although it had a much lower viscosity in the whole range, with 2000 cP at 10 °C and 14 cP at 44 °C. This tendency could be attributed to the introduction of 1-butanol, which acted as a co-surfactant for both PEO and PPO. 1-butanol could dissolve both PPO and PEO and also had some solubility in water [17,51]. The change in hydrophobicity of the solvent mixture shifted the sol-gel transition to an even lower temperature for a given triblock polymer concentration. Thus, it was already in the gel-like state for the solution of 22% F127, 61% TBE and 17% 1-butanol (wt%) even at a temperature as low as 0 °C. The gel-to-solution transition could also be observed at the investigated temperature region (less than 60 °C). This gel-to-sol transition could be ascribed to the decrease in solubility of both PPO and PEO chains (in water) with increasing temperature. The decrease led to less hydrated PEO blocks, resulting in less overlap by PEO fringes with a smaller interfacial area per polymer chain and a decrease in viscosity at elevated temperatures. These changes finally resulted in losing the close-pack order that permits the polymer solution to become a freely flowing fluid again (homogeneous molecular mixture [52]). The gel-to-sol transition of a series of solutions composed of Pluronics F127 in TBE/1-butanol was examined, with partial data listed in Table 1. Another point worth mentioning is that the significant difference in the viscosity between these two solutions, as shown in Figure 1A and 1B, is at least partially, if not wholly, coming from the different nanostructures formed (hexagonal or lamellar domains). The viscosity issue plays a key role in designing separation matrices for DNA separation by microchip electrophoresis.

3.2 SAXS

SAXS has been widely used to investigate the nanostructures formed by triblock copolymers in the gel-like state [47,53] and is a powerful technique for determining nanostructures and their corresponding lattice parameters. Previous studies have shown that for the binary system formed by F127/TBE solution within the concentration range of 21.2% to 28% (w/v), a face-centered cubic packing of spherical micelles could be formed in its gel-like state.

Figure 2A displays the 1-D circular integration profile (A), representing the scattered intensity of 22% F127, 61% TBE and 17% 1-butanol (wt%). Using the Bragg diffraction peak positions, it is therefore confirmed that the F127 gel structure in TBE/1-butanol at this concentration is a 2-D hexagonal close-packing of cylinders (HCPC). The observed relative peak positions produced the typical ratio with 1: (3)^{1/2}: (4)^{1/2}: (7)^{1/2}. Likewise, the corresponding diffraction planes can be indexed as (1 0), (1 1), (2 0), (2 1).

From the s (scattering vector) value at the first order peak, the inter-planar spacing (d) can be calculated by using the Bragg equation

$$d = 2\pi / q = 1 / s \quad (1)$$

with $q = \left(\frac{4\pi}{\lambda} \right) \sin\left(\frac{\theta}{2}\right)$, representing the magnitude of the momentum transfer vector. The relation between the cell edge of a hexagonal packing of cylinders (a) and Bragg spacing (d) is

$$a = \frac{2}{\sqrt{3}} d \quad (2)$$

and the radius of the cylinder (R_c) is related to intra-particle scattering by [54]

$$R_c q_n = 5.03, 8.364, 11.585 \quad \text{for } n=1, 2, 3 \quad (3)$$

From the above three equations, we can calculate the Bragg spacing d to be 16.8 nm, the dimension of unit cell as $a = 19.3$ nm, and the radius of cylinders to be about 5.0 ± 3 nm by taking $q_1 = 2\pi s_1 = 2\pi \times 0.16 = 1.0$ nm. This computation agrees with the fitting results ($a = 19.3$ nm, and $R_c = 4.7$ nm), using the Bessel function. The volume fraction of PPO cylinders ϕ can be estimated by

$$\phi = \left(\frac{2\pi}{\sqrt{3}} \right) \left(\frac{R_c}{a} \right)^2 \quad (4)$$

to be 0.24, assuming that only PPO blocks resided in the core. All the data are listed in Table 3. In this sample, the PPO core had a higher volume fraction (0.11) when compared with 28.2% (w/v) F127 gel in TBE buffer (0.078), which had a FCC parking pattern with $a = 26.3$ nm and $R_c = 4.4$ nm [26]. Note that the volume fraction of FCC can be calculated from a and R

$$\phi_{FCC} = \left(\frac{4\pi}{3} \right) 4 \left(\frac{R}{a} \right)^3 \quad (5)$$

It may be due to the presence of 1-butanol, the co-surfactant that has led to a slightly looser core.

The orientation of the cylinders can be demonstrated by the azimuthal (radial) profile at a desired radial position. Here the radius was chosen at the first peak (1 0) and the corresponding profile is shown in Figure 2B. It is likely that the hexagonal packing of cylinders is preferred to be orientated under the measurement conditions.

Interestingly, to alter the weight fraction of 1-butanol will induce the change of micellar structures. The SAXS scattering pattern of 22% F127, 50% TBE and 28% 1-butanol (wt %) was obviously different from the 2-D hexagonal packing we discussed above. From the peak position ratio denoted on the 1-D scattering intensity profile (Figure 3A), it was identified as a lamellar structure with the peak position as 1 : 2 : 3. The Bragg spacing can be calculated to be 17.7 nm, using equation 1. The experimental data are listed in Table 2. Thus, three different nanostructures were obtained with a fixed weight percentage of F127 by tuning the weight fraction of the second solvent (1-butanol) in the buffer solution.

The azimuthal (radial) profile at the radius of the first order peak was obtained and shown in Figure 3B. A four-point preferred orientation was present in the 1-D lamellar structure, compared with the two-point orientation in 2-D hexagonal. Moreover, the degree of preferred orientation of these 1-D layers was even higher than that of the 2-D cylinders, as indicated in Figure 2B.

A schematic diagram of the nanostructures, together with their scattering position ratios [53] is shown in Figure 4. Another feature of these nanostructures was that they did not exhibit a significant change in structure when replacing the pure water with 1×TBE. Only a small variation was observed on the structure parameters [26].

3.3 Nanostructured matrices used as DNA separation media

Separation performance—Three key criteria, those of high sieving ability, low viscosity, and dynamic coating ability, are used to evaluate the separation media for DNA separation by capillary electrophoresis. It requires a relatively high polymer concentration (above the overlap concentration) for hydrophilic polymer solution to form a robust network, where the separation will take place. However, as high polymer concentrations will result in high viscosities for most polymer solutions, it becomes difficult to inject such a solution into the capillary. Replacing the separation medium afterwards also presents similar difficulties. Electro-osmotic flow is another problem associated with capillary electrophoresis. As pre-coating of a capillary involves multiple steps, it is difficult to obtain stable and reproducible coating by pre-treatment even if cost is not a consideration.

Pluronics gels have a unique sol-gel/gel-sol transition over the temperature range from 0 °C to 80 °C. These gels have therefore become desirable materials for application as separation media in DNA electrophoresis. Extensive studies have been carried out to optimize CE conditions and to improve separation performance. However, to our knowledge, as much of the previous work was focused on the cubic structure, no reports on other ordered structures have been published. Herein, we investigated the sieving performance of F127 with 2-D hexagonal packing and 1-D lamellar structures in the gel-like state. As discussed above, the F127/TBE/1-butanol with the weight fraction of (0.22/0.61/0.17) or (0.22/0.50/0.28) was in the gel-like state at room temperature, but became a free flow fluid at ~50 °C. Thus, we first heated the polymer solution up to a temperature above 50 °C, injected it, cooled it down to room temperature, and then ran capillary electrophoresis. A ten-minute resting time was given before each run so as to allow the polymer solution to complete self-assembly into equilibrium ordered structures without considering the orientation of domain structures.

Figure 5 demonstrates the separation of double-stranded DNA in CE by using F127 in TBE/1-butanol at room temperature, along with that in TBE as a comparison. The peaks up to base 1353 were resolved in less than 15 minutes in the 22%F127/61%TBE/17% 1-butanol (wt%), as denoted on the graph. The migration time of DNA fragments up to base 609 was comparable to that recorded when moving the same DNA fragments in the matrix formed by 22% (wt %) F127 in TBE buffer under similar conditions. It took about 11 minutes for base 609 to pass through both of these separation media with a separation length of 7 cm. However, four more minutes were required for base 1353 to migrate through the same distance in the matrix with 2-D HCPC, and only two more minutes were needed for that in 3-D FCC, giving a total of 15 min for base 1353 in HCPC and 12 min for FCC. In other words, the DNA mobility in these nanostructured matrices was not linearly proportional to the size of DNA fragments (Figure 6). Obviously, the size dependence of DNA mobility was stronger in HCPC, when compared with that in FCC. On the other hand, no effective separation was observed by using the gel with a lamellar structure (data not shown). The requirement for high orientation of a lamellar structure along the capillary channel could be a possible reason for its failure. However, we have not yet tried moving DNA fragments specifically across the interfacial boundaries in the lamellar nanostructure.

The running conditions of effective DNA separation, using F127, or the mixture of F127 and F87, are listed in Table 2, along with the corresponding size of DNA. Primary data showed that the composition of F127/TBE/1-butanol with 2-D hexagonal structure resulted in better resolution than that of F127/TBE (FCC) when separating Φ X174 DNA-Hae III digest (Figure 5). However, the CE conditions have yet to be optimized for both of these two matrices. Our previous data showed that F127/TBE can be used to separate the oligonucleotides in the range of 8 to 32 bases, plus the dsDNA, by using a short separation channel length of 3 cm (Table 3). A second copolymer (i.e. F87) will change the mesh size, allowing a channel length as short as 1.5 cm. Our next step is to test our new ternary system in separating the oligonucleotides. The existence of organic solvent (here 1-butanol) could act as a denaturant in ssDNA separation so as to reduce the amount of urea or other denaturing agents.

Mechanism of DNA separation by Pluronics copolymers—The mechanism of electrophoretic migration of DNA in Pluronics gels is different from that found in conventional gels, e.g., polyacrylamide gels [55]. In the cubic quasi-lattice, there are at least four qualitatively different domains formed by the self-assembling of the triblock copolymer in gel state, as denoted in the diagram (Figure 7), including (1) condensed P or B micellar cores, (2) hydrated E micellar shells, (3) entangled E chains in the overlapping micellar shells between the closest micelles, and (4) solvent-rich interstitial gaps between micelles.

As listed in Table 2, the size of the PPO cores was very small for all these ordered structures, with 4.6 nm for FCC, 4.7 nm for 2-D hexagonal, and 4.0 nm for BCC. On the other hand, the center-to-center distance between the nearest neighboring micelles in the cubic structures or the cylinders in the 2-D hexagonal was much larger than the core size. The value was essentially the same within the experimental errors for FCC (19.1 nm) and 2-D hexagonal (19.3 nm), with a smaller value for BCC (10.7 nm). Highly charged DNA molecules should avoid hydrophobic micelle cores and migrate through hydrated poly(ethylene oxide) meshes and interstitial domains [25]. The larger “shell” areas could be providing more space for DNA to pass through. However, even those hydrophilic regions are not spacious enough for big molecules. A possible explanation for why dsDNA could be effectively separated in these media is that when they (Φ X174 DNA with a radius of gyration of 170 nm) are migrating in the gel, the PEO bridges (3 in Figure 7) will break down and be pushed away from each other by the DNA, leading to a temporary change in the lattice structure. Nevertheless, the resistance is expected to be very strong, because the

gaps between neighboring PPO cores are much less than the size of DNA, especially in BCC formed by 40% (w/v) B20-5000 with an even shorter center-to-center distance. Thus, the DNA coils have to be deformed to be extended against the strong obstructions. The longer the DNA chain, the more extended the chain becomes, leading to a non-linear size-dependent migration, in agreement with the electrophoretic behavior discussed above. Single-stranded DNA is more flexible. For instance, the hydrodynamic radius of 30-mer oligonucleotides is only about 1 nm. So it is more difficult for them to destroy the nanostructure of the sieving media. Instead, they are expected to move through the existing pores. The more dense hydrophilic regions (2 in Figure 7) formed by intramolecular PEO entanglements may account for their separation; and the separation is just like that of HPLC in this respect, the only difference being in the driving force. These two different mechanisms may be a possible explanation for the puzzle that the dsDNA and oligonucleotides showed similar mobility in this type of separation media [36].

On the other hand, the cylinders in the HCPC are more difficult to be slid away and give more resistance to the movement of big dsDNA molecules, leading to a better resolution for larger fragments at the expense of migration time and stronger size dependence of DNA mobility, as shown in Figure 5 and Figure 6. In other words, an HCPC structure may be preferred for the separation of larger DNA fragments, though more experiments are required to confirm this conjecture. In addition, 2-D HCPC is anisotropic, while cubic structures are isotropic. The orientation should be another consideration when performing in 2-D HCPC (or 1-D lamellar). The direction and the degree of preferred orientation will essentially determine the DNA migration curves in the gels. We have not yet observed good separation in highly orientated 1-D lamellar, probably because of the lack of control on the oriented lamellar domains. Further tests are required in order to draw a firm conclusion. In particular, a real-time *in-situ* synchrotron SAXS experiment could be helpful in explaining the whole process. It can track the orientation *in vivo* as well as the structure deformation of these Pluronics gels along the capillary channel during the CE process. A confocal image tracking of DNA deformation will be of use to watch the deformation and movement of DNA molecules.

4. Concluding Remarks

Pluronics gels, due to their unique thermal assembly behavior in a single solvent or two selective solvents, have been widely studied and applied in many areas, including biomedical and biological fields. Typically, at an appropriate concentration, they are free flow fluids at low temperatures but turn into a gel-like state at elevated temperatures and are accompanied by a sharp increase in viscosity. The system can often be transformed back into a solution state with further increase in temperature. SAXS has been applied to confirm the nanostructures formed by F127 triblock polymer in TBE buffer with or without 1-butanol, a co-surfactant for PEO and PPO. The scattering results have been used to determine the lattice parameters.

One of the noticeable applications of Pluronics copolymers is its possible utilization as a sieving medium in DNA capillary electrophoresis. It can be easily injected into the capillary due to its thermal switchable viscosity at an appropriate temperature. This merit becomes even more important when applied to microchips. Other advantages include dynamic coating ability and low toxicity. Double-stranded DNA separation was investigated by using different types of quasi-lattice nanostructures other than the cubic structure. As a demonstration of the concept, Φ X174 DNA was, for the first time, successfully separated in a 2-D hexagon structure, although the performance of the separation has yet to be optimized further.

The separation of DNA was essentially performed in the space between the PPO cores. The bridges formed among PEO chains of neighboring micelles have to be distorted to allow the long dsDNA fragments to pass through, while the smaller single-stranded oligonucleotides can simply migrate through the existing pores of the nanostructures. No effective separation was observed in the lamellar domains formed by F127 in a 28% (wt %) 1-butanol.

Abbreviations

BCC	body-centered cubic
BNL	Brookhaven National Laboratory
DMA	<i>N,N</i> -dimethylacrylamide
EB	ethidium bromide
FCC	face-centered cubic
HEC	2-hydroxyethyl cellulose
HCPC	hexagonal close-packing of cylinders
LPA	linear polyacrylamide
NMF	<i>N</i> -methylformamide
NSLS	National Synchrotron Light Source
PEO	Poly(ethylene oxide)
PPO	Poly(propylene oxide)
SAXS	small angle X-ray scattering

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Fig. 1.

Plot of viscosity vs. temperature of 22% (wt%) F127 in TBE/1-butanol at different weight fractions of 1-butanol. A, 22%F127/61%TBE/17% 1-butanol; B, 22%F127/50%TBE/28% 1-butanol.

Fig. 2.

Plot of 1-D circular integration profile representing the scattered intensity of the gel formed by 22% F127, 61% TBE and 17% 1-butanol (wt %) at 10 °C (A) and the azimuthal (radial) profile at the first order peak (B).

A

B

Fig. 3.

Plot of 1-D circular integration profile representing the scattered intensity of the gel formed by 22% F127, 50% TBE and 28% 1-butanol (wt %) at 10 °C (A) and the azimuthal (radial) profile at the first order peak (B).

BCC:

$$1:(2)^{1/2}:(3)^{1/2}:(4)^{1/2}:(5)^{1/2}:(6)^{1/2}$$

FCC:

$$(3)^{1/2}:(4)^{1/2}:(8)^{1/2}:(11)^{1/2}:(12)^{1/2}:(16)^{1/2}$$

HCPC:

$$1:(3)^{1/2}:(4)^{1/2}:(7)^{1/2}:(9)^{1/2}:(12)^{1/2}$$

LAM:

$$1:2:3:4:5:6:7$$

Fig. 4.

Electropherograms of Φ X174 DNA-Hae III digest by using the matrix formed by (a) 22%F127/78%TBE and (b) 22%F127/61%TBE/17% 1-butanol (wt%). DNA was injected for 5 seconds at 300 V/cm. Measurements were carried out at 200 V/cm and room temperature. Total capillary length = 10 cm with an effective length of 7 cm and ID = 50 μ m. Peak assignments are as indicated in (a).

Fig 5. Mobility of different size DNA fragments in different media by (a) 22%F127/78%TBE and (b) 22%F127/61%TBE/17% 1-butanol (wt%). Running conditions were the same as in Fig. 4.

Fig. 6.

A schematic diagram denoting the formation of micellar gels with the grey chains denoting the PEO ends and the orange chains representing PPO blocks. a, unimer; b, single micelle; c, micelles overlapped to form a network.

Fig. 7.
Diagrams of the ordered nanostructures and their peak position ratios. [53]

Table 1

Gel-to-sol transition temperature of Pluronics copolymers in TBE/1-butanol.

Composition	Gel at 24 °C	Gel-to-sol transition (°C) ⁽¹⁾	Structure reported at 25 °C [17]
15% F127, 54% TBE, 33% 1-butanol	Yes	40 °C	Lamellar
17% F127, 73% TBE, 10% 1-butanol	Yes	52 °C	Cubic
18% F127, 58% TBE, 24% 1-butanol	No (gel at 0 °C)	/	Micellar
22% F127, 61% TBE, 17% 1-butanol	Yes	52 °C	Hexagonal
37% P123, 60% TBE, 3% 1-butanol	Yes	70 °C	Hexagonal

¹ Gel-sol transition temperature range was broad. The temperature indicated was the finishing temperature

Table 2

Unit cell parameters of nanostructures formed by F127 or B20-5000 in TBE buffer with or without 1-butanol.

Composition of gel	21.2% F127 in TBE (w/v)[26]	28% F127 in TBE (w/v)[26]	40%B20-5000 in TBE (w/v)[28]	F127/TBE/ 1-butanol (22%/61%/ 17%) Wt %	F127/TBE/ 1-butanol (22%/50%/ 28%) Wt %
d (nm)	17.0	15.6	8.8	16.8	17.9
q (nm ⁻¹)	0.369	0.403	0.716	0.374	0.351
a (nm)	29.5	27.0	12.4	19.3	
R _c (nm)	4.6	4.6	4.0	4.7	
Center-to-center distance (nm)	20.9	19.1	10.7	19.3	
Nanostructure	FCC	FCC	BCC	2-D Hexagonal	Lamellar

Table 3

List of separation conditions for F127 matrices. For oligonucleotides, injection was done at 300 V/cm for 1 s; for Φ X174 DNA-Hae III digest, injection was done at 300 V/cm for 5 s.

Matrix	Separation length (cm)	Electric field (V/cm)	Temp	DNA	Migration time (min)
23% (wt%) F127in TBE buffer	3 cm	800	Room temp.	Oligonucleotide sizing markers ranging from 8 to 32 base	1.3 min for base 32
23% (wt%) F127+ F87 (in TBE buffer	1.5 cm	300	44 °C	Oligonucleotide sizing markers ranging from 8 to 32 base	2.6 min for base 32
22% (wt%) F127in TBE buffer	7 cm	200	Room temp.	Φ X174 DNA-Hae III digest	12 min for base 1353
22%F127/61 %TBE/17%butanol (wt%)	7 cm	200	Room temp.	Φ X174 DNA-Hae III digest	14 min for base 1353