A Review of Electrophoretic Separations in Temperature-Responsive Pluronic Thermal Gels

Cassandra L. Ward,[1,2]* Mario A. Cornejo,[1] Shakila H. Peli Thanthri,[1] and Thomas H. Linz[1]*

[1]Department of Chemistry, Wayne State University, Detroit, MI

[2]Lumigen Instrument Center, Wayne State University, Detroit, MI

*Corresponding Authors

Dr. Thomas H. Linz
ORCID 0000-0002-9517-901X
Wayne State University
5101 Cass Ave, Detroit, MI 48202
Email: tlinz@wayne.edu
Phone: 313-577-2580

Dr. Cassandra L. Ward
ORCID 0000-0001-6736-6769
Wayne State University
5101 Cass Ave, Detroit, MI 48202
Email: ward@wayne.edu
Phone: 313-577-2587

Abstract

Gel electrophoresis is a ubiquitous bioanalytical technique used in research laboratories to validate protein and nucleic acid samples. Polyacrylamide and agarose have been the gold standard gel materials for decades, but an alternative class of polymer has emerged with potentially superior performance. Pluronic thermal gels are water-soluble polymers that possess the unique ability to undergo a change in viscosity in response to changing temperature. Thermal gels can reversibly convert between low-viscosity liquids and high-viscosity solid gels using temperature as an adjustable parameter. The properties of thermal gels provide unmatched flexibility as a dynamic separations matrix to measure analytes ranging from small molecules to cells. This review article describes the physical and chemical properties of Pluronic thermal gels to provide a fundamental overview of polymer behavior. The performance of thermal gels is then reviewed to highlight their applications as a gel matrix for electrokinetic separations in capillary, microfluidic, and slab gel formats. The use of dynamic temperature-responsive gels in bioanalytical separations is an underexplored area of research but one that holds exciting potential to achieve performance unattainable with conventional static polymers.

Contents

4. Concluding Remarks

Abbreviations

BGE	Background electrolyte
bp	Base pair
CE-SSCP	Capillary electrophoresis single-strand conformational polymorphism
CGC	Critical gelation concentration
CGE	Capillary gel electrophoresis
CMC	Critical micelle concentration
CMT	Critical micellization temperature
dsDNA	Double-stranded DNA
EOF	Electroosmotic flow
GE	Gel electrophoresis
HPLC	High-performance liquid chromatography
ITP	Isotachophoresis
LOD	Limit of detection
MGE	Microchip gel electrophoresis
nt	Nucleotide
PDMS	Polydimethylsiloxane
PEO	Polyethylene oxide
PPO	Polypropylene oxide
SANS	Small-angle neutron scattering
SAXS	Small-angle X-ray scattering
SDS	Sodium dodecyl sulfate
SGE	Slab gel electrophoresis
ssDNA	Single-stranded DNA
T_g	Temperature of gelation
tITP	Transient isotachophoresis

1. Introduction to Gel Electrophoresis

Electrophoresis is a well-established technique for separating biological molecules (e.g. nucleic acids, proteins) in capillaries or microfluidic channels [1-4]. Analyses are conducted by first filling the separation space with a conductive background electrolyte (BGE) solution. Sample is then injected and voltage applied to induce analyte ions to migrate in the electric field. The migration rate of each analyte is

dependent on its charge-to-size ratio. More highly charges species experience higher Coulombic attraction to the oppositely charged electrode. Larger species experience higher frictional drag forces as they migrate through the BGE, proportional to their hydrated radii [5, 6]. The combination of opposing Coulombic attraction and frictional drag forces determines the electrophoretic mobility of an analyte. Although small molecules can often be separated based on mobility differences, the relative differences in charge and cross-sectional areas between different biological macromolecules (e.g. nucleic acids, proteins) are often insufficient to resolve distinct species. To overcome this problem, polymers are added to the BGE at sufficiently high concentrations to form solid, porous gels. Gels increase frictional drag to accentuate size differences between analytes and increase separation resolution [7, 8].

Gel electrophoresis (GE) is generally conducted within slab gels comprised of either polyacrylamide or agarose [9-11]. The gel polymers provide a network of pores through which biomolecules can transit. Analytes entangle in the gel to different degrees during the electrophoretic separation to enhance differential migration and promote separation. Pore sizes are controlled by the concentration of polymer used to cast the gel. Agarose gels are comprised of 1–8% agarose. These gels are not crosslinked, but rather rely on the entanglement of polymer chains to create pores 100–500 nm [8, 12]. Agarose gels are typically used to separate nucleic acids (0.1–20 kbp) [8] and large proteins (>200 kDa) [13]. Polyacrylamide gels contain 4–20% acrylamide along with a crosslinking agent to form covalently crosslinked pores 20–200 nm in diameter [10]. Polyacrylamide gels are well-suited for separating short nucleic acids (<5 kbp) [8] and proteins (5–200 kDa). For both polymers, gel pores are fixed once the gel is cast, so it is important to select the correct polymer concentration—and, consequently, pore size—to separate the analytes of interest [14-16]. Any biomolecules in a sample that are too large for the pores remain trapped at the top of the gel and cannot resolve from similarly sized species. Conversely, analytes that are too small migrate through the gel with minimal interaction with the polymer and cannot resolve from similar-size species. To maximize interactions of diverse analytes with the gel, gradient gels are commonly used that decrease in pore size from top-to-bottom of the gel. Gradient gels accommodate analytes over a wider range of molecular weights to better help validate biological samples [9, 17, 18].

The ubiquity of slab gel electrophoresis (SGE) in biological research laboratories stems from their relatively low cost ($15 per gel) and modest run times (1 h). However, analytical measurement scientists often avoid gels because of their relatively low separation efficiencies and mediocre detection limits. A major cause of this limited analytical performance is the low voltages that can be applied to slab gels (5 V/cm) [19, 20]. Operating at such low electric fields is necessary to avoid succumbing to the detrimental effects of Joule heating (e.g. air bubbles, smeared bands) [21]. However, performance is reduced because separation efficiency is directly proportional to the applied voltage. Conducting GE in a capillary or microfluidic channel, rather than a slab gel, mitigates this problem. These low-volume separation systems

reduce electrophoresis current and more efficiently remove heat from the system [22, 23]. This enables higher fields to be applied (200–1000 V/cm) to decrease analysis time and increase separation efficiency, which cannot be achieved in the slab format [24-26]. Furthermore, capillary gel electrophoresis (CGE) and microchip gel electrophoresis (MGE) require 100-fold less analyte mass (100 pg) compared to slab gels (10 ng), which facilitates analyses of precious biological samples [27, 28]. Capillary and microchip formats also afford quantitative measurements using more sensitive detectors to improve limits of detection (LODs) compared to gel scanners [29-32]. Absorbance detection is commonly coupled to CGE to monitor analytes as they migrate past the detection point. The intrinsic absorbance of proteins and nucleic acids enable label-free detection. Fluorescence is commonly employed in MGE to provide high sensitivity detection, even with the short pathlengths afforded by microfluidic channels. Although sample preparation steps are typically required to fluorescently label biomolecule analytes, this detection scheme provides superior LODs (sub-nM) than other techniques.

Although CGE and MGE provide efficient separations of biomolecules, practical experimental constraints have limited adoption of these techniques in biological research laboratories. For example, simply loading gel into capillaries and microchannels is tedious and time-consuming. This inconvenience is compounded by the need to remove gel after the analysis. Flexibility in analytical performance is also limited, as it can be difficult to tune gel properties to achieve optimal separations of the target analytes. Thermal gels, however, are an attractive alternative to conventional gels. These temperature-responsive polymers enable the physical properties of the gel to be controlled dynamically. While several polymers offer these benefits [33-48], this review focuses on the Pluronic family of polymers because of the unique benefits they provide for electrophoretic separations. The ability to reversibly convert Pluronic thermal gels between liquid and solid states and tune their sieving properties using temperature, buffer, and solvent provides unrivaled versatility for bioanalytical measurements. The following sections provide the reader with background information on the physical and chemical properties of Pluronic thermal gels. Literature is then reviewed which employed thermal gels for biomolecule separations in CGE, MGE, and slab gel formats. Applications from the last 30 years are discussed to highlight the advantages of thermal gels compared to traditional agarose and polyacrylamide gels. While Pluronic has been previously reviewed [49-55], this is the first review discussing its unique properties in the context of electrophoretic separations. Applications that do not employ Pluronic for the separation or that use other gel polymers are outside the scope of this review and are not discussed. Although temperature-responsive Pluronic gels remain an underexplored area of separations science, their numerous benefits highlight their potential utility in bioanalytical research.

2. Properties of Pluronic Thermal Gels

Pluronic polymers (non-proprietary name "poloxamers") were first developed in 1950 by BASF [49, 56, 57]. Initially manufactured for their use as emulsifiers and detergents, Pluronics are now commonly used in a variety of applications including cell culture, pharmaceutical drug delivery, and cosmetic products [50, 51, 56-63]. Pluronic polymers are non-ionic triblock copolymers comprised of polyethylene oxide (PEO) and polypropylene oxide (PPO). These copolymers have the formula $(PEO)_x(PPO)_y(PEO)_x$, where x and y subscripts indicate the number of hydrophilic PEO and hydrophobic PPO subunits (Figure 1). Numerous Pluronic formulations are commercially available that differ in the ratio of PEO:PPO, such as F127, F87, and P123. The naming scheme for specific Pluronic polymers provides information regarding the polymer structure, which is designated by one letter and two or three digits [50]. The letter indicates the physical state of the polymer at room temperature: liquid (L), paste (P), or flake (F) [56]. The first digit—or first two digits—indicates the approximate molecular weight of the PPO region, after multiplying by 300. The last digit provides the percentage of PEO content in the polymer, after multiplying by 10. As an example, Pluronic F127 begins with an "F", indicating that the polymer is a solid flake at room temperature. The "12" indicates that the molecular weight of the PPO content is ~3,600 g/mol (i.e. 12 x 300). Finally, the "7" indicates that PEO comprises 70% of the polymer's total subunits. Comparing F127 and P123, both compounds have an equal molecular weight of the hydrophobic PPO segment, represented by the "12", but F127 has a larger percent of hydrophilic PEO content (i.e. 70%) than P123 (30%). The relative amounts of hydrophobic and hydrophilic subunits influence the physical form of the polymer and the properties they exhibit in a thermal gel solution. Table 1 lists several Pluronic polymers that have been used in electrophoretic separations along with their physical and structural properties.

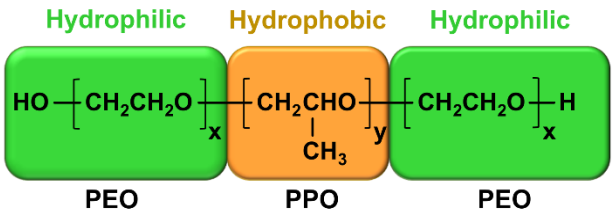

Figure 1. Pluronic thermal gels are triblock copolymers comprised of polyethylene oxide (PEO, green) and polypropylene oxide (PPO, orange).

Table 1. Chemical and physical properties of several different Pluronic polymers $(PEO)_x(PPO)_y(PEO)_x$ used in electrophoretic separations

Pluronic	Molecular weight[a]	Average number of PPO units (y)[a,b]	Average number of PEO units (x)[a,b]	CMC (μM)[c] at 37 °C	CMC (%w/v) at 37 °C	CMT[d] (°C)	Micelle shape[e]	Core size radius (nm)[e]	Corona thickness radius (nm)[e]	Packing lattice[f]	Cloud point (°C)[g]
F68	8400	29	76.3	480	0.40	40.0	Spherical	2.1	5.1	Cubic BCC	>100
F87	7700	40	61.3	91	0.070	29.4	Spherical	3.0	NA	NA	>100
F88	11400	39.3	103.9	250	0.29	30.5	Spherical	2.5	6.4	Cubic BCC	>100
F108	14600	50.3	132.7	22	0.032	24.5	Spherical	2.8	7.7	Cubic BCC	>100
P123	5750	69.4	19.6	4.4	0.0025	12.5	Cylindrical	4.2	2.4	Cubic to Hexagonal	90
F127	12600	65.2	100.2	2.8	0.0035	19.5	Spherical	3.8	7.0	Cubic FCC	>100

[a]Average unit size is based on average molecular weight provided by BASF
[b]PPO: polyethylene oxide; PEO: polypropylene oxide
[c]Critical micelle concentration (CMC) reported at 37 °C, pH 7.4 using a fluorescent probe (pyrene) technique [64]
[d]Critical micelle temperature (CMT) reported at 5 % (w/v) aqueous solution [65, 66]
[e]Calculated at 25 °C [67] or 20 °C [68]
[f]BCC: body-centered cubic; FCC: face-centered cubic [69-73]
[g]Cloud point for a 1% aqueous solution [49]
NA = not available

2.1. Micelle Formation and Gelation

The amphiphilic character of non-ionic Pluronic copolymers gives rise to interesting physical properties. Individual Pluronic molecules—called unimers—self-assemble into micelles when dissolved in aqueous solution, similar to other surfactants. Two important physical properties describe the self-assembly process: the critical micelle concentration (CMC) and the critical micelle temperature (CMT). The CMC and CMT values are the concentrations and temperatures, respectively, at which thermodynamically stable micelles form (Table 1). Lower CMC and CMT values generally indicate more favorable micelle formation. The CMC and CMT for a given Pluronic decrease exponentially with increasing PPO content of the triblock copolymer, indicating that PPO drives micellization, as discussed below. The PEO content does not influence the CMC/CMT [73].

The CMC is temperature dependent, and as temperature increases, the CMC for Pluronic F127 decreases by ~10-fold per 5 °C [65, 72]. Similarly, the CMT is concentration dependent, and as concentration increases, the CMT shifts to lower temperature [73, 74]. At Pluronic concentrations (c) below the CMC (when $c < CMC$), insufficient numbers of unimers are present in solution to associate into micelles, even at high temperatures. However, when the polymer concentration is above the CMC ($c > CMC$), a critical number of unimers is present for self-association into micelles when above the CMT. A particularly interesting property of Pluronic polymers is when the concentration is sufficiently large ($c >> CMC$), increasing temperature causes a transition from a liquid-phase mixture of micelles and unimers to a solid-phase gel comprised of micelles packed into an ordered crystalline lattice [49, 75]. The polymer concentration at which this gelation occurs is called the critical gel concentration (CGC).

For a Pluronic solution where $CMC < c < CGC$, the association of unimers into micelles involves an equilibrium between three temperature regions: unimer, transition, and micelle (Figure 2A) [74]. Below the CMT, the solution is predominantly made up of unimers. When the temperature reaches the CMT, PPO units of the unimers begin to dehydrate and aggregate with other unimers. This produces micelles that have a hydrophobic core of dehydrated PPO units and a hydrophilic corona of solvated PEO units. The CMT marks the beginning of the temperature region called the transition. The transition region is relatively broad because polydispersed unimers and micelles exist in a dynamic equilibrium over a wide temperature range [73, 74, 76]. The concentration of micelles increases linearly in this region with temperature until all the unimers have self-associated into micelles ($T > CMT$).

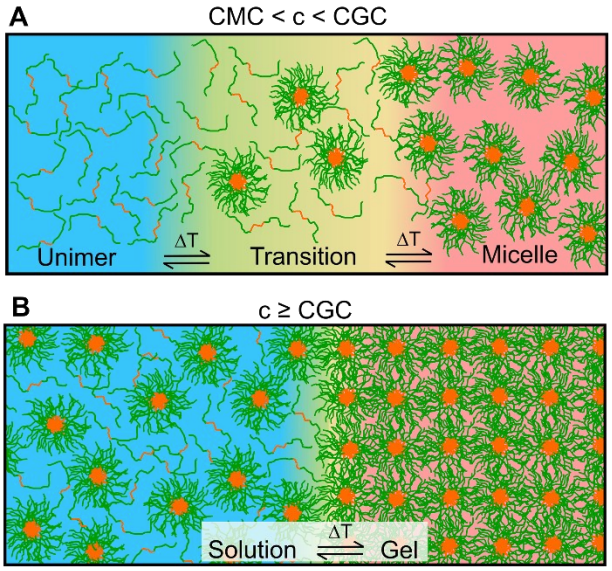

Figure 2. Illustrations of temperature-dependent Pluronic structures in aqueous solutions. **(A)** When CMC < c < CGC, three regions can form depending on the solution temperature, labeled as unimer (blue), transition (yellow), and micelle (red). The concentration of micelles increases as temperature increases, but the micelle concentration is not high enough to form a solid gel. **(B)** When c ≥ CGC, Pluronic polymers can be used as thermal gels. Solution-phase micelles and unimers (blue) are present at lower temperatures, but micelles readily assemble into close-packed solid gels (red) above the gelation temperature (yellow). Green: PEO; Orange: PPO; CMC=critical micelle concentration, CGC=critical gelation concentration.

Pluronic solutions form thermal gels when c ≥ CGC, which solidify at the gelation temperature (T_g). The CMT and T_g are different parameters, where T_g > CMT because micelles must first form above the CMT before a critical number is reached at a higher temperature to pack into a solid gel. Pluronic thermal gels exhibit a sharp transition from liquid solutions to solid gels as temperature increases (Figure 2B), unlike the broad transition region when polymer concentrations are lower (Figure 2A). This abrupt transition into a gel state arises from micelles being forced together beyond the point of hydrodynamic contact, where the intermicellar distance is at or less than the micelle diameter [71]. Micelles undergo periodic ordering that is driven by repulsive forces to maintain distance between the hydrophobic PPO cores [73]. This micelle packing also creates physically entangled networks of solvated PEO chains.

The packing of Pluronic micelles has a significant effect on the viscosity of thermal gels. Figure 3 shows viscosity curves for different concentrations of F127 in water [77]. At low temperature, Pluronic thermal gels are free-flowing Newtonian fluids regardless of polymer concentration. The viscosity of these liquid-phase gels decreases slightly with increasing temperature, similar to typical liquids [78, 79]. At the CMT, however, viscosity increases due to an increase in the concentration of micelles. As temperatures

increase further, significant changes occur in the gels, depending on the polymer concentration. At concentrations below the CGC (e.g. 12.5%), relatively small increases in viscosity are observed (10-fold) starting at the CMT; however, the concentration of micelles is too low for an ordered thermal gel to form [77, 80]. At higher polymer concentrations (e.g. 20%), micelles pack densely above the CMT. Viscosity increases substantially once the T_g is reached (10^8-fold), as an ordered solid gel forms [77]. The T_g decreases with increasing Pluronic concentration because more micelles are present to readily pack into a gel (Figure 3) [78]. Increasing the concentration of Pluronic also increases the maximum viscosity of the solidified thermal gel [77, 79, 81]. The ability to control thermal gel viscosity using temperature and polymer concentration provides unrivaled versatility for electrophoresis analyses. Temperature can be adjusted in real-time to load liquid-phase gel into a capillary or microchannel and then increased to solidify the gel to the desired viscosity. This dynamic behavior also enables analyte migration through the thermal gel to be tuned to maximize resolution in bioanalytical separations, which will be discussed further in Section 3.

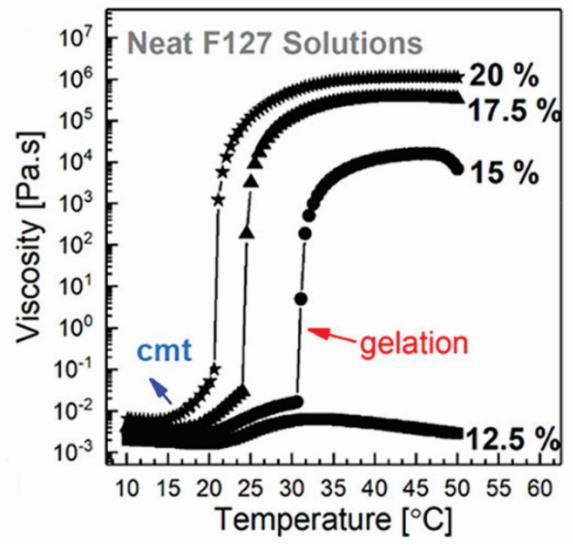

Figure 3. Viscosity curves of different F127 concentrations in water demonstrate that thermal gels are highly dependent on temperature. The CMT value corresponds to the initial increase in viscosity. The T_g (labeled as gelation) is the sharp inflection point in the viscosity curve. Figure adapted from Ref. [77].

Another interesting property of Pluronic thermal gels is their optical transparency regardless of the temperature, concentration, or the phase of the polymer [82]. Most polymers have relatively low cloud points where the solution becomes opaque because the polymer is no longer soluble at certain temperatures and concentrations. This opacity obscures optical detection of analytes in the gel. Pluronic thermal gels, however, have cloud points well above normal running conditions (>100 °C, Table 1). Because the phase

of the thermal gel has no bearing on its optical transparency, thermal gels are viable for a broad range of bioanalytical applications irrespective of the required analysis temperature.

2.2. Micelle and Lattice Structures in Gel Packing

Pluronic thermal gel micelles have different shapes and sizes in solution depending on the polymer concentration, temperature, and specific Pluronic used (Table 1). Various light scattering techniques have been used to characterize micelles as either spherical, cylindrical, or lamellar (Figure 4A). The PEO:PPO ratio of the polymer influences micelle shape; larger ratios (>0.5) predict spherical micelles, while smaller ratios predict cylindrical micelles (~0.25) or lamellar micelles (~0.1) [73]. The shape of the micelles dictates the mesophase of the gel. Spherical micelles form a cubic phase; cylindrical micelles form a hexagonal phase; lamellar micelles form a sheet-like lamellar phase (Figure 4A) [83].

Temperature-dependent dynamic light scattering (DLS) has shown that at low Pluronic concentrations (CMC < c < CGC), unimers have a hydrodynamic radius of ~1 nm and micelles have a radius of ~10 nm [49]. Solidified thermal gels (c > CGC) have been analyzed using small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS), which found the radius of the micelle PPO core (R_{core}) to be 3–5 nm and the intermicellar distance (D_{inter}) to be 15–18 nm [70-73, 80, 81]. The specific sizes of R_{core} and D_{inter} are dependent on the specific Pluronic type. However, the general trend for all Pluronic polymers is that D_{inter} is only affected by the polymer concentration, not temperature, where distance decreases with increasing concentration [71-73]. This occurs because more micelles must be packed within the same unit volume, thus forcing the micelles into closer proximity. Once a thermal gel is in a solid state, R_{core} and D_{inter} are generally unaffected by further increases in temperature because the PPO core is already completely dehydrated (Figure 4B) [72, 73, 80, 81]. However, the PEO corona length (R_{corona}) decreases with increasing temperature due to dehydration of the PEO chains ($R_{corona}^{T_{warm}} > R_{corona}^{T_{hot}}$). PEO continues to dehydrate and collapse towards the micelle core until a sufficiently high temperature is reached where the micelles can no longer maintain a closed-packed structure and the thermal gel reverts back to a solution phase [80]. However, this temperature is above those conducive for electrophoretic separations of biological molecules.

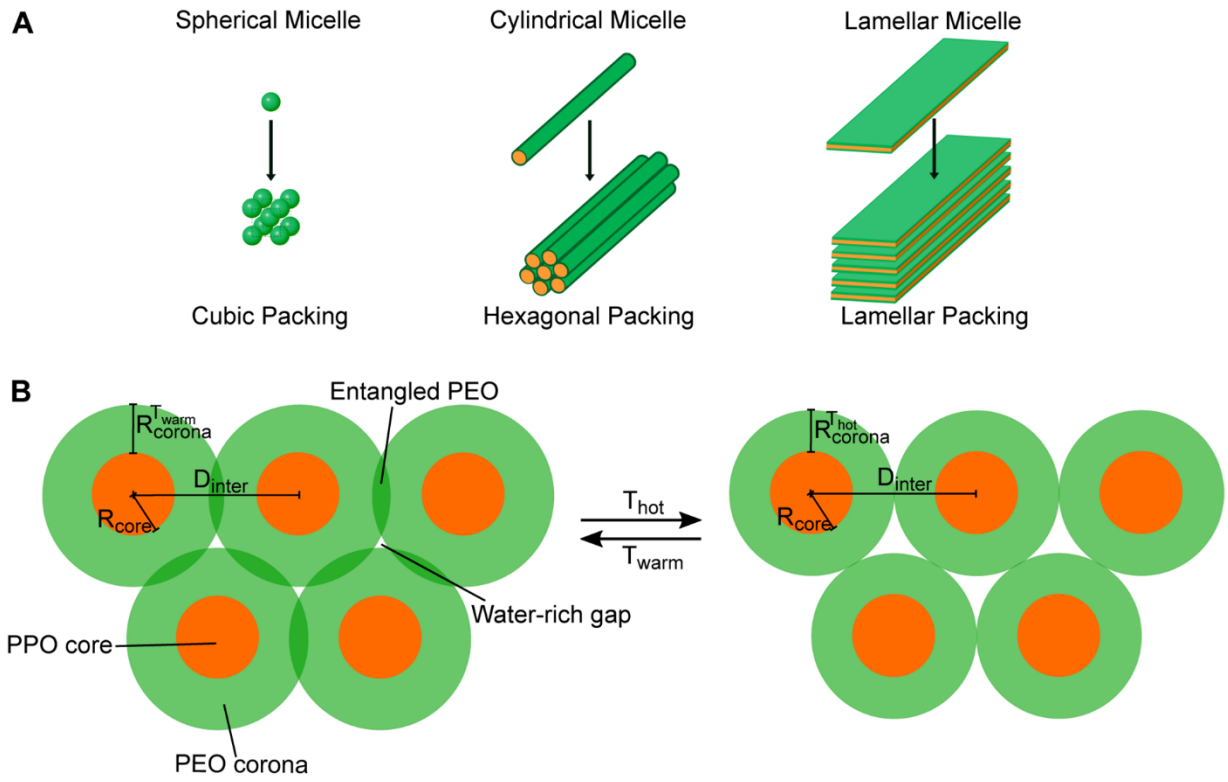

Figure 4. (A) Cartoons illustrating the different micelle shapes and their respective mesophase gel packing structures. **(B)** The face-centered cubic (FCC) structure of F127 thermal gel, where c > CGC and T > T_g. The microstructure involves four distinct regions: hydrophobic PPO micelle cores (orange), hydrated PEO coronas (green), entangled PEO regions (dark green), and water-rich gaps. As temperature increases (T_{hot}), PEO units become dehydrated and shrink towards the core $\left(R_{corona}^{T_{hot}} < R_{corona}^{T_{warm}}\right)$. R_{core} and D_{inter} do not change with temperature. Thus, higher temperature increases the size of water-rich gaps (i.e. pores), providing more space for analytes to migrate. Figure based on results from several references [72, 78, 84-86].

The mesophase that Pluronic micelles pack into is a consideration when using thermal gels as a matrix to separate biomolecules. The lattice packing structure influences analyte migration through the gel. Most thermal gels used for electrophoretic separations (Table 1) form spherical micelles (PEO:PPO >0.5) that pack into an isotropic cubic structure. This packing structure creates four distinct regions: hydrophobic PPO micelle cores, hydrated PEO coronas, entangled PEO regions, and water-rich gaps (referred to as pores) (Figure 4B) [78]. During electrophoresis, analytes will migrate following the path of least resistance through the open pores before entangling with the deformable PEO coronas or being forced into the more rigid micelle cores. A benefit of thermal gels is that the interactions between the analytes and the gel can be tuned using temperature to control the mesophase packing. As previously described, SAXS and SANS

experiments show the PEO chains shrink towards the micelle core with increasing temperature, while maintaining the D_{inter}. The shrinking of the PEO chains with unchanging D_{inter} results in wider pore regions through which analytes can transit. The use of temperature provides a simple mechanism to dynamically control thermal gel pore sizes and influence separation outcomes.

The types of mesophases that form are dependent on temperature and polymer concentration, which can be mapped out in phase diagrams (Figure 5). For example, the phase diagrams for F127 and F68 show that isotropic phases—mixtures of micelles and unimers—occur at lower temperatures and concentrations; this indicates that the thermal gel is in a liquid state. As temperature increases, F127 and F68 solidify as they reach the 2Φ line (2Φ indicates an intermediate state between two phases) and form cubic structures. Note that gelation occurs at much higher temperatures and concentrations for F68 than F127. The lower hydrophobic PPO content of F68 makes micellization—and ultimately gelation—less favorable. Thus, high temperatures and polymer concentrations are required to solidify F68 thermal gels, diminishing their practicality for the analysis of biomolecules. P123, however, only requires moderate temperatures and concentrations to form spherical or cylindrical micelles (Figure 5), which increases its versatility. In fact, P123 can exist in multiple mesophase structures in aqueous solution that are readily interchangeable. Cubic structures form at lower temperatures and concentrations, whereas hexagonal and multi-phase structures (mixture of cubic and hexagonal) occur at higher concentrations and temperatures. As P123 micelles convert from cubic to hexagonal packing, the micelle core size and D_{inter} increase [66, 87], which influences analyte migration through P123 thermal gels.

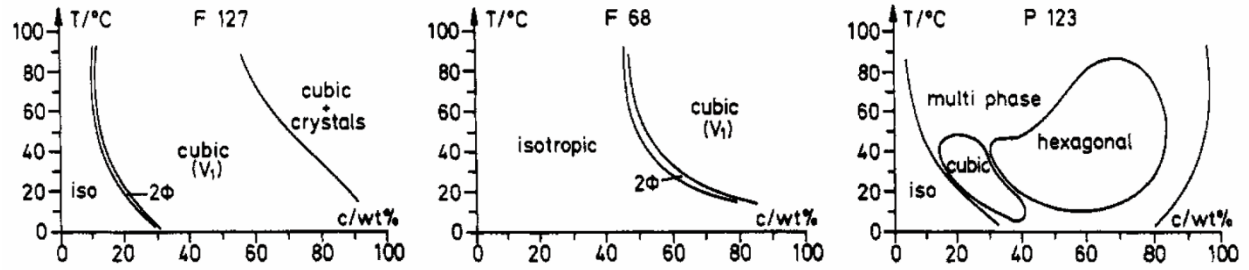

Figure 5. Phase diagrams of F127, F68, and P123 indicate the mesophase that forms at specific temperatures and polymer concentrations. Multiphase is a mixture of different phases, 2Φ is an intermediate state, and iso is the isotropic state of a mixture of liquid micelles and unimers. Figure adapted from Ref. [73].

2.3. Effects of Buffer Modifiers

Electrophoresis requires a conductive BGE to carry out a separation, so the effects of buffers and salts on the T_g and viscosity of thermal gels are important considerations when designing separations

experiments. The addition of ionic species generally shifts thermal gel T_g values to lower temperatures. When salt is added, water molecules preferentially hydrate the salt ions, consequently dehydrating the polymer. This causes the effective polymer concentration in the thermal gel solution to increase [88], thus lowering the CMT and T_g. For example, the addition of NaCl to 1% (w/w) F127 lowers its CMT by 12 °C/M NaCl [89]; but the hydrodynamic radius of micelles is unaffected, as shown by DLS experiments [90]. Because salt affects the viscosity of the gel, the operating temperature of an electrophoresis analysis may need to be adjusted for optimal performance. Added salts also decrease cloud points of Pluronic solutions. For example, the cloud point of 1% (w/w) F68 decreases from >100 °C to 50 °C by adding 1 M KF [90]. Similarly, the cloud point for 30% (w/w) F127 decreases 44 °C/M NaCl [88]. However, as most electrophoresis experiments utilize <100 mM concentrations of salt, the cloud point will not have a significant effect on the transparency of the gel, so analytes will remain readily detectable. The pH of the buffer can also impact gel solidification. Gelation of 30% F127 and F87 were measured across a pH range [91]. Although the gelation temperature for F87 was unaffected by pH, F127 did not solidify below pH 6 even at temperatures up to 70 °C; F127 in acidic buffers remained in an isotropic transition state. The reason for this is unclear and may simply be an artifact of the salts used to make the buffers, as salts decrease the T_g, as described above.

Additions of sodium dodecyl sulfate (SDS), urea, and organic solvents are common when preparing biological samples for electrophoresis. These reagents exhibit an effect on the T_g of thermal gels, but often counter to those produced by salts [88]. SDS is a surfactant that associates with Pluronic to form mixed micelles that require higher temperatures to solidify. Additionally, increasing concentrations of SDS cause micelles to undergo a significant reduction in size [92]. For 1% F127, light scattering experiments showed that the effective hydrodynamic radius of the micelle decreased from 11 nm to 5 nm with 1 mM SDS [93]. Above 2 mM SDS, only 2 nm molecules remained, approximately the size of one F127 unimer. SDS is thought to adsorb onto the hydrophobic PPO subunit of Pluronic, which prevents the unimer from aggregating with other unimers. Thus, addition of SDS decreases micellar aggregation and requires higher temperatures to solidify. Gelation is precluded at concentrations >110 mM SDS for 25% F127 [94]. Urea has the same effect on Pluronic micellization as SDS, but under a different mechanism. Urea molecules interact with the hydrophilic PEO units and the surrounding water, maintaining a hydrated unimer [95]. This prevents the dehydration of the unimers, requiring higher temperatures to initialize micellization. The effects of urea are more pronounced for Pluronic polymers with higher PEO content.

Addition of alcohols to Pluronic thermal gels affects the CMT, T_g, and cloud point with a dependence on the alkyl chain length. Short chained alcohols (methanol and ethanol) favor demicellization (increases CMT, T_g, and cloud point) while longer chain alcohols (butanol and larger) induce micellization (decreases CMT, T_g, and cloud point) [96-98]. Propanol is the intermediate where low concentrations of

propanol favor demicellization, but ≥20% favor micellization [96]. The degree to which the CMT, T_g, and cloud point shifts is dependent on both the concentration and the length of the alcohol's alkyl chain. NMR studies found that short chained alcohols disrupt the hydrogen bonding network around the micelle, thus more water molecules are available to prevent dehydration of the PPO core [98]. Longer chained alcohols create favorable hydrophobic interactions with the PPO core, expelling water from the core and inducing micellization [98].

The addition of salts and organic solvents can affect the gel mesophase formed for some Pluronic polymers that have multiple mesophases, like P123. A SANS experiment showed the time-dependent transformation from spherical to cylindrical micelles with 1 M NaCl for 1% P123 [87]. This occurs because the addition of salt increases the effective concentration of the polymer, meaning the transition line from cubic mesophase to hexagonal phase in the phase diagram is shifted to lower concentrations (Figure 5). Adding salt to F127, which only has one mesophase (Figure 5), shifts the gelation 2Φ transition line to lower concentrations, but does not create any new mesophases. However, additional phases can be created with the addition of organic solvents, such as butanol. The addition of butanol induces a new phase diagram for F127 that includes hexagonal and lamellar phases with the addition of 20–50% butanol [99].

The additions of buffers, salts, and modifiers to the BGE or sample affect Pluronic thermal gels and therefore should be considered when designing experiments. However, most electrophoretic separations are carried out under mild conditions, so salt and pH effects on the gel are relatively minor. The T_g shifts slightly to lower temperatures at <100 mM NaCl, while the cloud point remains >90 °C for the Pluronic polymers listed in Table 1. This enables thermal gels to readily facilitate electrophoretic separations of biomolecules at biologically appropriate conditions, as discussed in Section 3.

2.4. Pluronic Polymer Blends

Different Pluronic formulations can be blended together to alter the physical properties of thermal gels such as the T_g and viscosity, which are important properties for a separations medium. The T_g of a blend is shifted between the T_g of the two parent Pluronic polymers, proportional to the mixing ratio [100]. For example, the T_g of 30% F87 and 30% F127 in TBE are 38.1 °C and 17.8 °C, respectively, while a 1:2 blend of 30% F87:F127 has an intermediate T_g of 27.1 °C [100]. Similarly, the T_g values of 30% F68:F127 blends increase with higher percentages of F68. In fact, because pure F68 solutions are unable to solidify, F68:F127 blends cannot form a solid gel at higher blend ratios (>20:80) [101]. Interestingly, though, rheology experiments found that even in a F68:F127 blend ratio that does not solidify, the viscosity is just as high as a blend that does solidify [101]. The rheology data shows a sharp transition in viscosity with temperature (similar to Figure 3) suggesting that at higher temperatures the polymer still undergoes a change in state. Such gels are described as being in a glassy-amorphous state where a high density of

micelles is present in solution to significantly increase the viscosity, but a crystalline lattice does not form [101].

3. Pluronic Thermal Gels in Separation Science

The properties of Pluronic thermal gels described in the previous section highlight many facets which can impact electrophoretic separations. Controlling parameters such as polymer concentration, buffer, and temperature dictates the micelle packing density and absolute viscosity of the thermal gel. In the following sections, a comprehensive survey of literature employing Pluronics as thermal gels and coating agents is discussed. This information is presented to highlight the operating conditions in past work that influence the separations of biomolecules. This review will provide insight to guide the reader to select analysis conditions most suitable for their own analytes of interest.

3.1. Pluronic Coatings

Although Pluronics form solid thermal gels under appropriate conditions, historically, their most common use in separations science has been as coating agents in CGE or MGE to improve reproducibility and separation resolution [102-109]. Silica capillaries and glass or PDMS microchannels generally possess negative surface charges during electrophoresis. This charge attracts cationic analytes from solution, causing surface adsorption. The undesired retention of analytes along the channel wall causes band-broadening and reduces separation efficiency and reproducibility. This problem can be mitigated by covalently modifying the surface to alter its charge or hydrophobicity using silane chemistry [110-112], but this process can be tedious and require specialized equipment. Pluronic polymers provide an alternative to rapidly passivate capillaries or channels as a practical, cost-effective means of preventing nonspecific adsorption.

Pluronics can serve as static coatings where the polymer adsorbs to surfaces via physisorption. The procedure simply entails conditioning a capillary or channel with a Pluronic solution and then rinsing it out. On hydrophobic surfaces (e.g. PDMS), the hydrophobic PPO regions form an adsorption layer to passivate the surface and prevent analyte adsorption [107]. On hydrophilic surfaces (e.g. oxygen plasma-treated PDMS, fused silica), both PPO and PEO regions interact with the surface leading to flat adsorption of the polymer. Separation efficiencies of a protein mixture increased up to 13-fold in capillaries statically coated with Pluronic F108 compared to uncoated capillaries because proteins were prevented from interacting with the capillary wall [105]. Pluronic can also be used as a dynamic coating by adding it directly to the BGE during electrophoresis at concentrations below the CGC [106, 113]. For example, 0.1% F127 prevented protein adsorption and was more effective than a polyacrylamide coating, even after several analyses within

the same capillary [114]. Compared to untreated channels, non-specific adsorption was reduced by 94% in Pluronic-treated channels.

In addition to reducing analyte adsorption, Pluronic coatings also greatly reduce electroosmotic flow (EOF) [105, 107, 114]. The adsorbed polymer disrupts the formation of a layer of mobile cations near the capillary surface. Consequently, the bulk fluid transport typically observed in capillary zone electrophoresis cannot be established. For example, the addition of 0.1% F108 decreased EOF by 60-fold compared to an uncoated capillary. The elimination of EOF also improves separation resolution by accentuating the relative mobility differences between analytes. Pluronic polymers with more PEO units provide the largest decrease in EOF and the highest increase in separation efficiency [105]. Comparing capillaries coated with P103 (30% PEO) and F108 (80% PEO), the F108 coating decreased EOF 10-fold more and increased separation efficiency of a model protein by 10-fold. This example highlights the benefits of reducing EOF to improve separations, but it should be noted that in cases where both cationic and anionic analytes must be measured, separate analyses will be required because the EOF will not be present to push both ionic species towards the detector.

3.2. Thermal Gel Loading and Operation

Pluronic polymers at low concentrations provide effective surface coatings, but thermal gels that form at higher concentrations (c > CGC) provide a unique material in which to conduct CGE and MGE. The dynamic nature of thermal gels greatly simplifies analytical workflows when compared to conventional static gels comprised of polyacrylamide or agarose. For example, casting a polyacrylamide gel in a microchannel first requires acrylamide monomers and crosslinking reagents to be loaded into the channel. This filled device must be exposed to UV light to form the gel. Unreacted reagents must then be flushed out before use in bioanalytical applications. This tedious process must be repeated for every run. Further driving up cost of this approach is the inability to remove the crosslinked gel after an analysis, so new capillaries or microdevices must be used. Agarose presents another challenging gel material for analytical separations because the polymer is only liquid at high temperature. Loading hot polymer solutions often leads to bubble formation and incomplete gel loading through the capillary as the gel cools into its solid form. Thermal gels present a much more attractive gel material that overcome the challenges associated with loading other gel polymers and avoids the toxicities of materials like acrylamide [84].

Thermal gels can be readily loaded into capillaries or microchannels by taking advantage of their temperature-dependent viscosity. At low temperatures (e.g. 10 °C), thermal gels exist in a liquid state. These low-viscosity solutions can be readily introduced into narrow channels or capillaries (≤100 μm diameter) by applying vacuum or pressure to load the gel or allowing capillary action to fill them [33, 85, 115]. Depending on the specific Pluronic used and its concentration (e.g. >20% F127), loading should be done

on ice or in a cold room to ensure thermal gel is maintained as a liquid. Once filled, the temperature can be increased (e.g. ambient temperature) to induce gelation. Converting the thermal gel into a solid state provides a sieving matrix for electrophoretic separations [116-118]. Because the viscosity of thermal gels is reversible, the gel matrix can be removed after an analysis by cooling the temperature and removing the liquid-phase gel. The ability to load and remove thermal gels is desirable for creating multiuse devices [85]. Unlike polyacrylamide, fresh Pluronic thermal gels can be reloaded into the same channel/capillary for multiple analyses, saving the device from being discarded and reducing overall analysis costs.

Although fresh gel can be loaded between analyses, it would be advantageous to reuse the same gel in multiple runs to expedite analyses. To evaluate whether this approach is viable, CGE was used to analyze replicate DNA samples on the same capillary filled with 21.2% F127 [78]. Results from this study revealed degradation of the separation with every sequential run, as migration times increased and separation current decreased. Interestingly, this problem could be overcome by leaving the capillary overnight to recondition. Migration times could also be recovered after applying a reverse electric field between runs. Performance degradation was attributed to polarization of ions within the gel network during consecutive runs. However, the authors concluded that the best way to regain separation performance was to replace the thermal gel after each run, which reduced the relative standard deviation to <2%. Thus, using fresh thermal gel is recommended for every analysis.

Thermal gels provide another advantage in their ability to spatially segregate electrolytes and analytes in different regions of a microfluidic device. Thermal gels with distinct compositions can be introduced into different channels within a microfluidic device at low temperatures. Increasing the temperature then solidifies the gels, preserving reagents in their intended discrete locations. One study monitored the diffusion of a small molecule dye through solidified F127 thermal gel between different microchannels. Only minimal diffusion was observed after 1 h, unlike in a control experiment without thermal gel (Figure 6) [119]. This demonstrates the ability of thermal gels to maintain concentration gradients of small molecules between adjoining gel regions. This spatial segregation is beneficial for techniques such as transient isotachophoresis (tITP) that require a discontinuous buffer system. tITP employs two pairs of electrolytes: one pair for analyte enrichment, one pair for electrophoretic separation. Thermal gel preserves these discontinuous buffer systems prior to analysis, greatly reducing diffusion and unwanted mixing that would otherwise preclude the analysis. The use of discontinuous electrolytes in thermal gels has been demonstrated in the tITP analyses of DNA and proteins in microfluidic devices [27, 119].

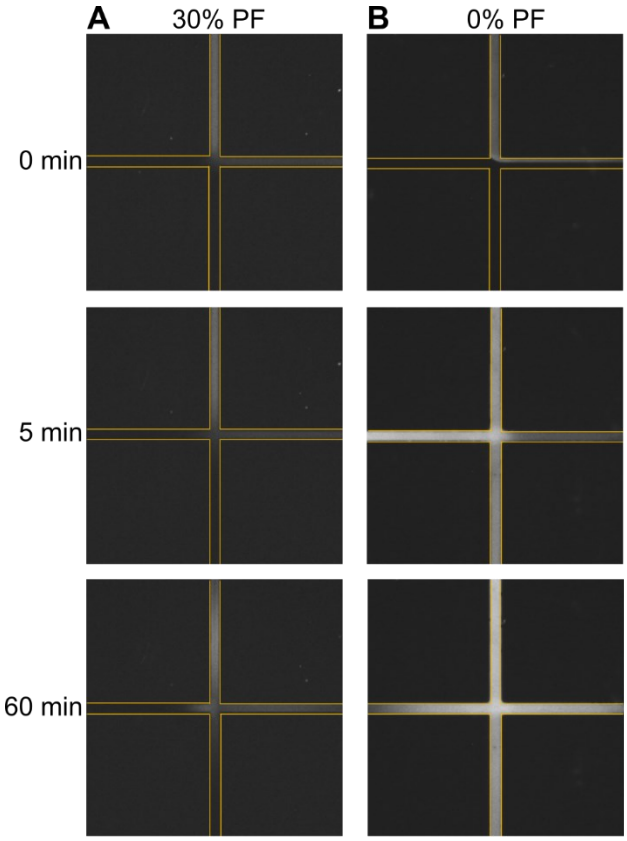

Figure 6. A cross-channel microfluidic device was filled entirely with **(A)** 30% F127 or **(B)** 0% F127 with the sample channel (top) and waste channel (right) also containing a small molecule fluorescent dye. Images are displayed 0, 5, and 60 min after channel filling. Thermal gel was found to minimize diffusion of the dye into the other channels even after an hour. Yellow lines highlight the channel boundaries. "PF" indicates the percentage of Pluronic F127 in the device. Figure from Ref. [119].

3.3. Nucleic Acid Analyses

The primary applications of Pluronic thermal gels in electrophoresis to date have been in the analysis of DNAs (Table 2). Although numerous studies have been conducted by international researchers, two main groups pioneered the field. The first demonstration using thermal gels as a separations matrix was published by the Chu group in 1997 [72] followed closely by the Rill group in 1998 [84]. Prior to this work, Pluronic was only used as a coating agent for capillaries, as described above. Studies from these two groups and others determined the effects of polymer concentration, temperature, type of Pluronic, and voltage on the separations of DNA and other biological molecules.

Chu and coworkers first demonstrated that solidified F127 (21.2% and 28.0% (w/v) in TBE) could be used to separate a DNA ladder (89 to 1560 base pairs (bp)) using CGE [72]. Narrow, well-resolved bands were observed using a 13 cm fused silica capillary applying 200 V/cm. An anomalous DNA migration was

found in the small bp region (138 and 157 bp fragments migrated in reverse order), which is consistent with SGE and CGE analyses using polyacrylamide and attributed to sequence-dependent conformations that affect DNA mobility [120, 121]. These initial results validated that separations in Pluronic thermal gels can be achieved comparably to those in conventional gels. Rill and coworkers then used tube gel electrophoresis and CGE to separate double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), and supercoiled DNA plasmids with F127 thermal gel [84]. dsDNA up to 3,000 bp was separated in 20% F127 in a 13 x 0.5 cm tube. Comparisons between tube gel electrophoresis using 20% F127, 2% agarose, and 6% polyacrylamide found that F127 thermal gel was superior for resolving smaller DNAs (246-615 bp DNA), while the resolving power was equivalent for 625-861 bp, and worse for >1000 bp. These early studies demonstrated that thermal gels are capable of analyzing nucleic acids similar to conventional gel polymers; however, the dynamic nature of the thermal gels have the potential to be harnessed to improve analytical performance.

Table 2. Applications and operating conditions for electrophoresis conducted in Pluronic thermal gels.

Analyte	Platform	Pluronic Type	Percent Pluronic (%)	Buffer	Temperature (°C)	Electric Field (V/cm)	Reference
ssDNA (8–32 nt, 255–456 nt)	Capillary, Glass microchip	F127, F87, F127:F87[a], F108, F68,	9–35	TBE[b] Proprietary buffer solution	20–50	200–600	[78, 83, 84, 100, 122-131]
dsDNA (10–3500 bp)	Capillary, Tube gel, Glass Microchip, PMMA Microchip, PDMS Microchip, Slab	F127, F108, P123, F127:F108[a], F108:F88[a], F108:F87[a], F127:F68[a]	13.5–30	TBE[b] TBE/1-butanol Aminocaproic acid and HCl, Proprietary buffer solution, Tris–HCl	15–60	CGE and MGE: 15–30, 177–500, 1200 Tube and Slab: 6–8	[72, 78, 83, 84, 86, 115, 119, 123, 126, 132-135]
DNA Plasmids (>3,000 bp)	Capillary, PMMA Microchip	F127	20–30	TBE[b]	25	300–500	[83, 84, 132]
RNA (22-32 nts)	Capillary, Glass microchip, PDMS microchip	F127	20, 30	TBE[b] Aminocaproic acid and HCl, Tris–HCl	25–30	100–400	[133, 136-138]
Peptides (<3 kDa)	Slab, Capillary	F127	15–24	Tris–HCl Phosphate buffer	21, 40	Slab: 28 CGE: 270	[139, 140]
Proteins (>5 kDa)	Capillary, PDMS microchip	F127	15–30	TBE[b] Tris–HCl Phosphate buffer	10–40	200–750	[27, 72, 140, 141]
Small molecules	Capillary, PDMS Microchip	F127	20–30	TBE[b] Tris–HCl	25–30	185–625	[83, 119, 136, 142]
Cells	PDMS Microchip	F127	30	Tris–HCl	10	333–1333	[143]

[a]The colon indicates a blend of two Pluronic polymers

[b]TBE = Tris–borate–EDTA buffer: 89 mM tris(hydroxymethyl)aminomethane, 89 mM boric acid, and 2 mM EDTA

3.3.1. Concentration and Temperature Studies on F127

Pluronic F127 is the most commonly used thermal gel in the literature, and its effects on separations have been well characterized. A study was conducted to determine the effects of F127 concentration on the separation of a dsDNA digest (72 to 1353 bp). The polymer concentration was incrementally increased from 13.5% to 28%. CGE separations at 25 °C were first able to resolve distinct DNAs using 17.4% F127, with 10 of 11 species resolved. Note that the 17.4% F127 concentration is just above the CGC (17%) under these conditions [78]. Below 17%, F127 did not form a solid gel, and was unable to resolve the DNAs. At 21.2% F127, all 11 peaks separated; however, further increasing the thermal gel concentration only increased DNA migration times and did not improve separation resolution. This trend was validated in other studies that found increasing the thermal gel concentrations above 20% led to a decrease in separation resolution [84, 134]. Increasing thermal gel concentration increases the micellar packing density and forces more interactions between the DNA and the gel, thus slowing migration and likely increasing band-broadening. Analyte mobility was found to decrease by 2-fold going from 21.2% to 28.0% thermal gel [72]. Collectively, these studies determined that an optimal thermal gel concentration is needed to maximize separation resolution, similar to standard gels. Insufficient gel concentrations do not provide enough interactions between the analytes and the gel to separate, while excess gel slows analyte migration and prevents resolution between similar sized species.

A DNA digest was analyzed while varying the temperature of 21.2% F127 from 15 to 60 °C. All 11 DNA fragments separated below the T_g (Figure 7) [78]. This demonstrates that a solid gel is not essential for DNA separation as long as a sufficiently high polymer concentration is used. However, baseline separation of the larger DNAs (872 to 1353 bp) was not achieved below the T_g [78]; but increasing the temperature to 25 °C solidified the thermal gel and resolved the larger DNA fragments. Further increasing temperature to 40 °C resulted in the comigration of 271 and 281 bp fragments but did not affect the large DNAs. Additional comigration of smaller DNAs (72–281 bp) also occurred at hotter temperatures (≥40 °C), which is consistent with another report [134]. At a constant thermal gel concentration of 21.2%, the mobility of the DNAs increased by 2-fold as temperature was increased from 25 °C to 50 °C [72]. However, micellar density does not change with temperature above the T_g, as demonstrated by SAXS and SANS experiments. Thus, the increase in mobility with temperature is likely due to a combination of higher thermal energy increasing the rate of DNA migration and larger pore sizes stemming from the shrinking of the PEO chains (Figure 4B). Wider pores result in lower entanglement between DNAs and the polymer, which decreases separation resolution. This effect is accentuated in small DNAs because they preferentially migrate through the pores and do not interact with the PEO, consequently leading to comigration at higher temperatures. Thus, lower temperatures are preferred for smaller DNAs even if the thermal gel is not in a

solid state [78]. Large DNAs are less susceptible to this decreased resolution at high temperature because they are too large to exclusively transit through the pores and must interact with the PEO regions.

In summary, polymer concentration and temperature can be used to control effective pore sizes in Pluronic thermal gels to tune DNA migration. Although pore sizes in agarose or polyacrylamide gels can be controlled through polymer concentration, temperature cannot be used to fine-tune separation outcomes, which demonstrates the utility of thermal gels. Although previous studies on Pluronic thermal gels have limited their work to isothermal analyses, integrating dynamic temperature control into the analysis has the potential to adjust performance on-demand to achieve optimal separation conditions for each analyte. This gradient analysis strategy—similar to those implemented for protein analyses (discussed in Section 3.4)—poses interesting opportunities for future studies.

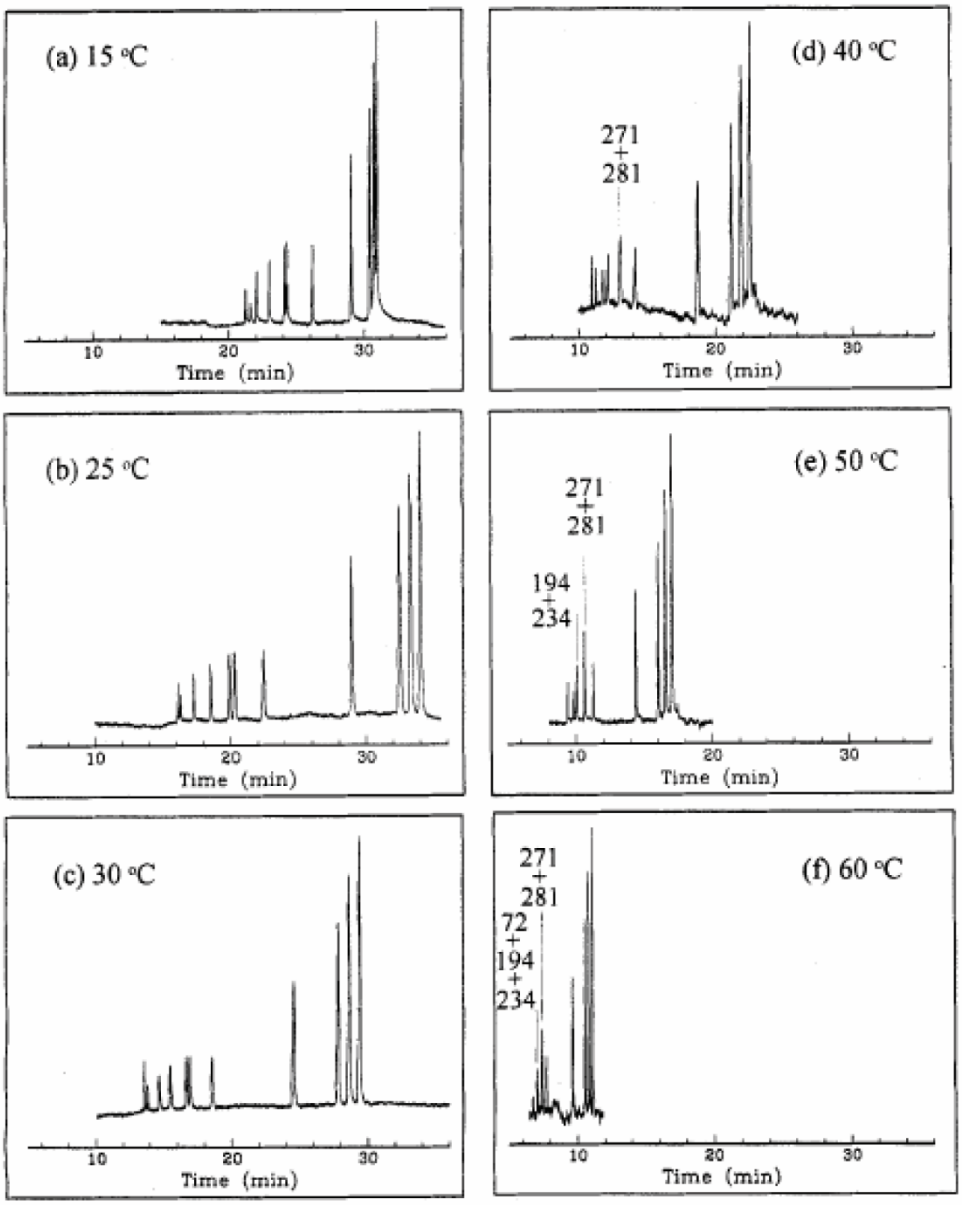

Figure 7. Electropherograms of a DNA digest using 21.2% F127 in TBE at various temperatures. Migration times and separation resolutions are affected by the temperature dependence of the thermal gel. Figure from Ref. [78].

3.3.2. Other Pluronic Thermal Gels

Several Pluronic thermal gels have been used to separate dsDNAs (e.g. F127, F108, F87, F88, and P123) [78, 86, 100, 126] with each having different effects on separation efficiency. However, there is a lack of systematic studies to determine which physical properties of Pluronic improve separation outcomes. Only one comparative study reported the resolution between two genomic DNAs of the same size (255 bp) in 15% F108, F127, F88, and F87 at 35 °C using capillary electrophoresis single-strand conformational

polymorphisms (CE-SSCP) [126]. Note that these thermal gel solutions were incapable of solidifying under the experimental conditions. High PEO content was reported to be a critical factor in achieving high resolution between DNAs. Given that the DNA strands interact with the PEO corona during migration, additional PEO composition likely accentuated entanglement of the polymer with one DNA conformation and, consequently, increased resolution between the pair. No trend was observed with increasing PPO composition, as expected, because DNA does not interact with the micelle PPO core. Interestingly, resolution was found to increase with increasing molecular weight of the Pluronic: F108 > F127 > F88 > F87. Further systematic studies directly comparing separation efficiencies of DNA over a broader size range in different Pluronic thermal gels are needed to validate conclusions.

3.3.3. Blended Pluronic Thermal Gels

Blends of two Pluronic copolymers have been utilized to improve separation efficiency of DNAs [100, 119, 126]. Higher resolution was attained using a blend of F87 and F127 fixed at 30% (w/v) when separating short ssDNA sizing markers 8–32 nt in microfluidic devices [100, 131]. Although the separation of these DNAs was previously achieved in unblended 30% F127 in a 3-cm long silica capillary [122], using 30% F127 in a 1.5 cm glass microfluidic device led to complete comigration of four of ten oligomers (Figure 8A) [100]. In 30% F87, only one of ten oligomers could be precisely quantified (Figure 8B). However, using a 1:2 blend of F87:F127 resolved all species (Figure 8C). Similarly, another study found that a 30% (w/v) blend of F68:F127 in a 1:9 ratio was optimal for separating a DNA ladder (50 to 1,000 bp) in a PDMS microfluidic device [119].

Studies have suggested that blending Pluronic copolymers with different PPO_1:PPO_2 ratios result in non-cooperative micelle formation (i.e. each Pluronic forms micelles only with itself) [144], which is suggested to be the case for F68:F127 [101]. A thermal gel containing 30% F68:F127 in a 1:9 ratio solidifies >27 °C [119]; however, based on rheology and calorimetry experiments, the gel consists of only F127 micelles with F68 unimers occupying the pores [101]. The unimers present in the pores may interact with the DNAs during electrophoresis and increase frictional drag to improve separation in a 1:9 F68:F127 blend compared to F127 alone [119]. More studies are needed to confirm this gel microstructure.

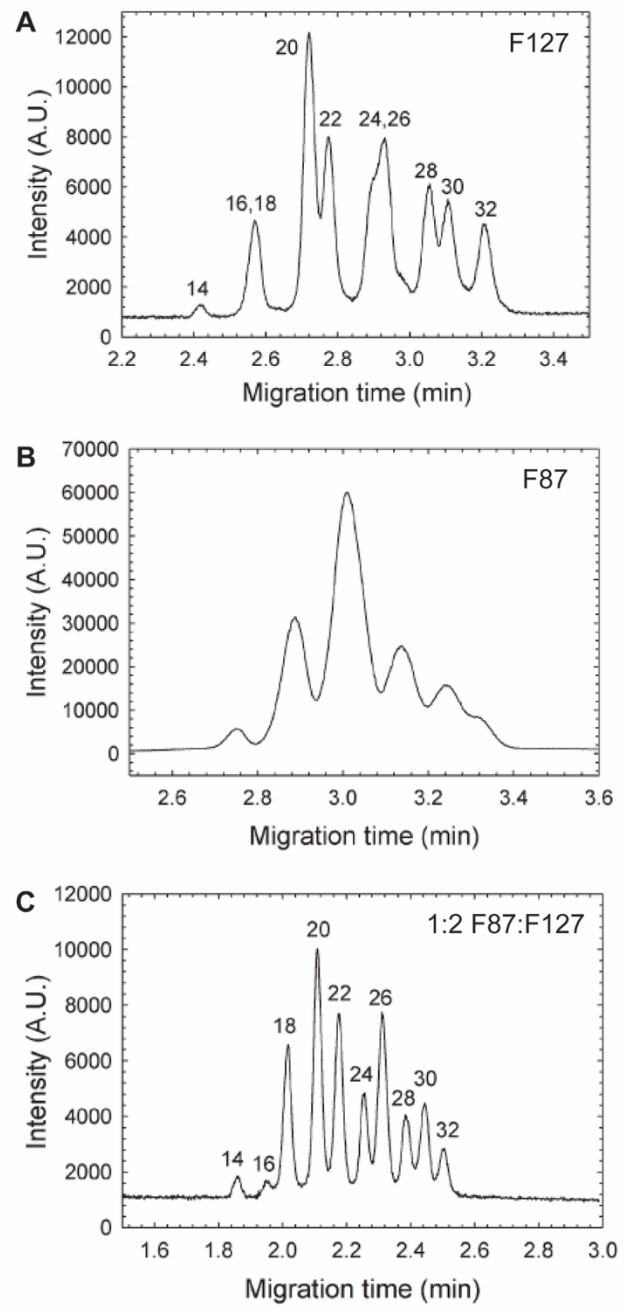

Figure 8. Electropherograms of ssDNA markers separated using **(A)** 30% F127, **(B)** 30% F87, and **(C)** 30% blend of F87:F127 in a 1:2 ratio. Figure adapted from Ref. [100].

Using Pluronic blends does not always result in increased separation performance. A CGE study evaluated Pluronic blends that had similar PEO content but spanned a large range of molecular weights. The highest separation resolution between two genomic DNAs (255 bp) was achieved in 15% F108. Resolution decreased when a blend was used, regardless of the ratio or type of Pluronic (F88, F87, or F127) added to F108 [126]. The blend that least effected resolution was F127 into F108, which could be attributed

to their similar molecular weights [126]. However, this is inconsistent with the studies described above that showed improvements when the molecular weights were appreciably different, e.g. 1:2 F87:F127 [100] and 1:9 F68:F127 [119]. There does not appear to be a systematic trend that indicates which Pluronic polymers should be blended to enhance separations of DNA. Evaluating the potential benefits provided by blends must be performed empirically.

3.3.4. Effects of Mesophases

Separations of dsDNA have mostly been conducted in Pluronic thermal gels that have cubic mesophases. Of the thermal gels listed in Table 1, only P123 can form hexagonal phase in aqueous solutions. However, the only study that used P123 in an electrophoretic separation maintained the thermal gel in the cubic phase for analysis [86]. F127 is generally in the cubic phase for all temperature and concentration ranges above the T_g and CGC (Figure 5). However, different types of mesophases can be induced in F127 by including butanol into the thermal gel [85, 99]. A DNA digest (72 to 1353 bp) was analyzed by CGE in 22% F127 in three different mesophases: cubic phase (0% butanol), hexagonal phase (17% butanol), and lamellar phase (28% butanol). No separation was observed in the lamellar phase, likely due to the lamellar sheets oriented perpendicular to the DNA migration, which impeded migration [85]. The hexagonal and cubic gel structures provided similar results, although a slight improvement in separation resolution was observed with hexagonal packing. The D_{inter} for hexagonal and cubic phases are the same within experimental error [85], which explains their similar separations performances. But in the cubic phase, DNA molecules can cause a dynamic change in the micellar structure as they migrate through the small pore regions, similar to any non-crosslinked gel (e.g. agarose). However, the cylinders in the hexagonal phase (Figure 4A) should be more rigid and better maintain their interactions with the DNA to improve separation resolution. Therefore, it is surprising that the hexagonal phase did not have a more pronounced effect on the separation efficiency. Further studies are needed to evaluate the effect of gel mesophases on larger DNAs, proteins, and other biomolecules. Studies using P123 would be especially interesting because cubic and hexagonal phases occur in aqueous conditions without requiring the addition of alcohol. However, maintaining thermal gel in a cubic phase likely helps reproducibility between replicate analyses because the isotropic packing of the spherical micelles has no orientational effects. This enables a capillary or microchannel to be reproducibly filled with uniformly packed micelles each time.

3.3.5 Mechanism of Migration

In traditional agarose and polyacrylamide gels, biomolecules must sieve through pores in the polymer matrix (20–500 nm), described by three models: Ogston, reptation, and reptation with stretching. The sieving mechanisms through traditional gels and other entangled polymers have been previously reviewed [8, 10, 145-147]. Generally, small DNAs (<1000 bp) follow the Ogston model where DNA

molecules migrate through the pores, colliding with the gel polymer chains as they travel. Larger DNA molecules (>1 kbp) follow the reptation model. Reptation involves a headfirst, snake-like motion of the analyte through the pores of a polymer network. Much larger DNA molecules stretch and become oriented in the direction of the electric field, which is termed the reptation with stretching model (other names include biased reptation or reptation with orientation). DNA molecules become stretched when hooked around obstacles (i.e. gel polymer chains) followed by relaxation back to the equilibrium conformation after sliding off the obstacle [8]. Studying DNA mobility as a function of size, gel concentration, and/or electric field strength informs the sieving mechanism.

Mobility studies of dsDNA were carried out to determine the sieving mechanism in Pluronic thermal gel. DNAs 118–1353 bp demonstrated linear Ferguson plots, which suggests Ogston sieving [78]. However, log-log plots of mobility vs. 1/bp showed a sigmodal shape for 17.7–28% F127 thermal gel concentrations, which is inconsistent with the Ogston model [78]. Similar plot shapes have been seen in other entangled polymer matrices (e.g. poly-N,N'-dimethylacrylamide) that were attributed to all three sieving mechanisms of separation occurring in a single analysis [8]. Although this could also pertain to thermal gels, the authors of the study concluded that the mechanism does not follow traditional sieving mechanisms because of inconsistencies between experimental results and each model [78].

Comparison studies were undertaken to determine the sieving mechanism of dsDNA 123–1800 bp in 20% F127, 5% polyacrylamide, and 2% agarose gels [84]. Results indicated that polyacrylamide and agarose gels followed the reptation with stretching model across the entire DNA size range. However, in F127, only the 246–738 bp DNAs obeyed the reptation with stretching mechanism [84]. Sequences outside of this size range deviated from traditional sieving models, similar to another report [78]. Another comparative study analyzing DNA (25–800 bp) in 20% F127 and methylcellulose observed a non-linear decrease in mobility with increasing size of dsDNA for both gels in MCE separations. This behavior indicates a fundamentally different mechanism than the previously observed reptation with stretching and the Ogston sieving typically observed with small DNAs [134].

The four regions in the micelle packing of Pluronic thermal gels should be considered in the elucidation of the separation mechanism (Figure 4B). The entanglement of the PEO coronas is non-crosslinked, similar to agarose gels. However, the crystalline packing of thermal gel micelles provide well-defined pore sizes at fixed temperatures and polymer concentrations, like crosslinked polyacrylamide gels. Because the micro-structure has elements of both agarose and polyacrylamide gels, the migration mechanism is likely a hybrid of both polymers [78]. Computational studies support this claim [86], showing that for dsDNA 20–3500 bp, the migration through FCC Pluronic gel matrices involves entanglement with high-density and low-density PEO regions and streamlined transit through the pores. The computational results agree with the experimental mobility results from a 2D-SGE study. DNAs 20–3500 bp showed an

initial rise in mobility up to ~100 bp in both F127 and P123 gels followed by a mobility decrease [86]. This occurs because small DNA molecules preferentially migrate through the pores and push away PEO as they move through. This forges a path for other DNA molecules, reducing the drag force and increasing mobility. This effect is observed up to a length of 100 bp at which point longer DNAs entangle with the PEO segments and migration slows [86].

Another consideration affecting the migration mechanism is that crystalline thermal gel does not form in one continuous domain inside a capillary, microchannel, or slab. Rather, several randomly oriented crystallites form with boundaries between each microstructured crystallite domain. Fluorescence imaging and linear dichroism studies demonstrated that these mesoscopic (micrometer scale) boundaries affect the migration of large DNA [117, 118, 148]. Under low electric field conditions (<10 V/cm), such as in SGE, large DNA migrates along the crystallite boundaries. However, under the high electric field strengths used in CGE and MGE (>15 V/cm), DNA causes reversable deformation of the gel microstructure as it migrates through the cubic lattices. More work is needed to determine the homogeneity of crystallites over a length scale relevant to separations (mm to cm). It remains unclear if more uniform packing would reduce boundaries and increase interactions with the gel to improve separations in low-voltage formats.

3.3.6 Other Nucleic Acid Analyses

The analyses of dsDNA and ssDNA is of primary interest to the biological research community, but methods are also required to analyze other classes of nucleic acids. For example, plasmids are key components required to obtain cellular expression of target proteins. Thermal gels facilitate validations of plasmid samples by enabling these large DNA sequences (2–10 kb) to be separated. Although thermal gels struggle to analyze linear dsDNA >3 kbp, the compact shapes of plasmids allow them to migrate through the gel [83, 84]. Additionally, ssDNA molecules of the same size can be separated by thermal gels. CE-SSCP has been used to separate same-length ssDNAs with different conformations[125-130]. DNAs derived from 16S rRNA genes were separated in 15% P108 to identify twelve strains of pathogenic bacteria [127]. This same separation could not be achieved with multiple standard gel polymers.

Short non-coding RNAs have also been separated using thermal gels. One study sorted short RNAs (~22 nts) from longer nucleic acids (>66 nts) using isotachophoresis (ITP) [133]. A trailing electrolyte was selected that enabled focusing of shorter, higher mobility RNAs while longer, slower RNAs could not enrich as they transited through the gel. Although individual RNA species could not be separated or identified with this approach, effective sample preparation was achieved to isolate target RNAs from other nucleic acids in cell lysates.

MicroRNAs (miRNAs) have been analyzed using a technique called thermal gel electrophoresis (TGE) developed by the Linz group [137]. TGE performs inline preconcentration and separation of

miRNAs without the need for sample injections or multiple fluidic channels. This performance is achieved by using thermal gel to spatially segregate molecules—both analytes and ITP electrolytes—in separate regions of single-channel microfluidic devices. The channel is entirely filled with sample, using the solidified thermal gel to preserve a discontinuous buffer system between the channel and reservoirs. Confining the sample to the channel and spatially isolating distinct electrolytes and maintaining concentration gradients enables hybrid ITP-MGE outcomes to be achieved (Figure 9A) [137, 138]. Four target miRNAs of the same length were enriched and separated using TGE (Figure 9B) in the analysis of cell lysate samples. Achieving analyte preconcentration followed by a self-initiated separation expedites analyses, while using low-complexity single-channel microfluidic devices lowers analysis costs. Selectivity of the analysis was subsequently enhanced by conducting the analysis at elevated temperatures [138]. Off-target interactions of three structurally similar miRNAs were eliminated by conducting TGE at 50 °C rather than 30 °C. Although electrophoresis generally cannot operate at high temperatures because of prevalent Joule heating concerns, the stability of Pluronic thermal gel at high temperatures enabled single-nucleotide resolution to be achieved between these homologous miRNAs.

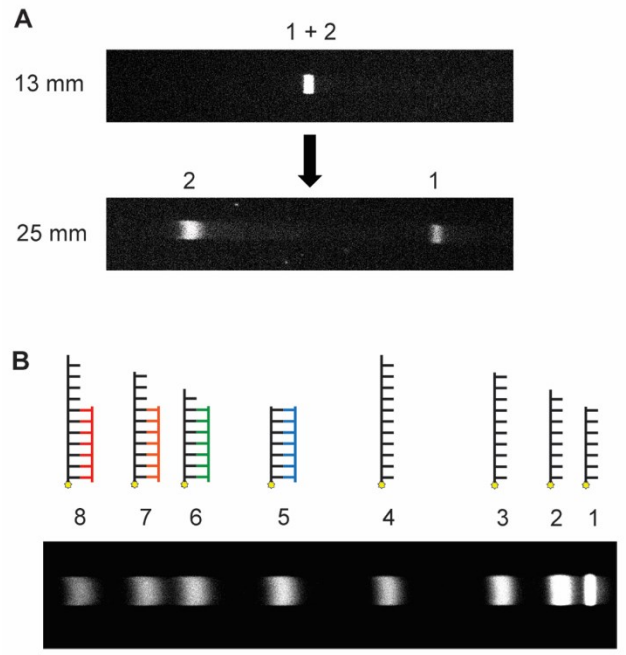

Figure 9. (A) A miRNA and its corresponding probe are loaded throughout the entirety of a single-channel microfluidic device. Sample enrichment is conducted (13 mm) followed by an automated separation (25 mm) of the single-stranded probe (Peak 1) and the double-stranded miRNA-probe hybrid (Peak 2). The thermal gel serves to both isolate electrolytes prior to analysis and act as a sieving matrix to help resolve analytes. **(B)** Four miRNA-probe hybrids separate from four excess probes. Figure adapted from Ref. [137].

3.4. Protein and Peptide Analyses

Thermal gels provide a separations matrix to resolve proteins and peptides with high efficiency. But even using low-concentration Pluronic polymers as coatings (c < CGC) provides a simple means of increasing method performance [104], as described in Section 3.1. Separation efficiencies of proteins and peptides increase when employing dynamic coatings of ≤10% Pluronic in a BGE [106, 113, 149]. A study reported that a CGE separation using 7.5% F127 produced higher resolution in a separation of collagen cyanogen bromide fragments (271 –27,000 Da) compared to separations performed using high-performance liquid chromatography (HPLC) [149]. Interestingly, increasing the Pluronic concentration to 18–30%, where a solid thermal gel is formed, was unsuitable due to long migration times (>2 h) and substantial peak dispersion. A 7.5% F127 dynamic coating was determined to be optimal [150]. The study also found that higher resolution was obtained at 20 °C than at 50 °C. This behavior can be attributed to higher diffusion at elevated temperature causing band-broadening and reducing separation efficiency.

Protein separations can also be conducted in thermal gels (c > CGC). The first electrophoretic separation of a protein in a solidified thermal gel was reported by Chu and coworkers who analyzed GH5 (9.6 kDa) in F127 using CGE [72]. The protein solution was electrokinetically injected into the gel, and peaks were observed from the protein and the excess small molecule labeling dye. Rill and coworkers separated myoglobin tryptic peptides (~2 kDa) using a 24% F127 slab gel and compared the separation to a 15% polyacrylamide gel [139]. The resolution between bands were comparable between the gels. The authors suggest sieving may occur in thermal gels as analytes squeeze between hydrated PEO regions, similar to sieving through polyacrylamide.

CGE studies were undertaken to determine the mass range of a series of model proteins (12.5–240 kDa) using a 7.5% F127 capillary coating [113]. Proteins >45 kDa did not elute after 1 h. Therefore, the polymer concentration was reduced to 5% F127 to increase analyte mobility. Separations of proteins 12.5–68 kDa under these conditions were superior to analyses without Pluronic. However, proteins >68 kDa still did not elute within a reasonable time frame (<1 h), and those that did experienced significant broadening [151]. The authors suggested that proteins >68 kDa become trapped by the thermal gel, despite insufficient amounts of polymer present to form a solid gel at 5% F127. Thus, an upper limit of ~50 kDa was set as a cut-off for realistic separations of proteins in Pluronic [106, 151]. An important observation from this study found multiple resolved peaks for hen egg albumin (45 kDa) from protein variants. This protein microheterogeneity could only be resolved with Pluronic added to the BGE. This highlights the improved resolution provided by Pluronic coatings to better assess fine protein structure [151].

The dynamic range of protein separations in solidified thermal gels was investigated by conducting tITP in PDMS microfluidic devices [27]. Proteins and protein subunits 6–464 kDa were separated in a fixed 10-min run time. Lower mass proteins (<50 kDa) migrated through the channel and were detected in the

time domain, as is standard in MGE. Larger proteins (>50 kDa) did not reach the detector within the analysis time, consistent with previous reports on the protein size limit in thermal gels [106, 151]. However, detection of proteins >50 kDa was achieved by incorporating a distance domain into the analysis (Figure 10). Voltage application ceased after 10 min, and the separation channel was imaged upstream from the detection spot (similar to SGE). This approach enabled large proteins to be measured within the same analysis as small proteins. Combining the time-domain electropherogram with the distance-domain electropherogram overcame the protein size limitation to facilitate analysis of proteins over a wide mass range.

Temperature gradients were also evaluated to enhance resolution between proteins in thermal gels. The optimized gradient decreased temperature from 35 °C to 25 °C during the analysis to decrease gel viscosity over time (Figure 10) [27]. This enabled low-mass proteins to separate under their optimal high-viscosity conditions and then provide time for high-mass proteins to separate under their optimal lower viscosity conditions. Again, thermal gels uniquely afford the ability to dynamically alter separation performance to attain optimal results.

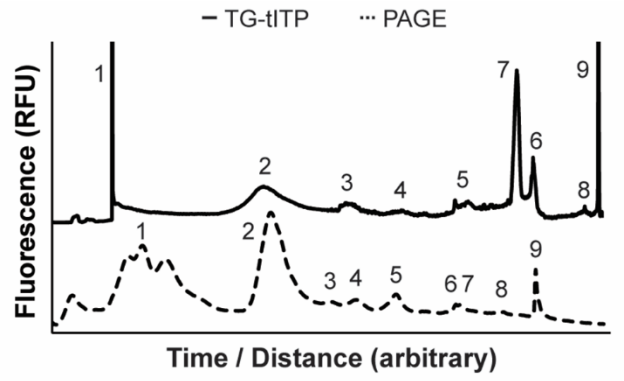

Figure 10. A sample containing nine proteins and protein subunits 6–464 kDa were analyzed by microfluidic tITP. Analyses in a F127 thermal gel using a 35 to 25 °C temperature gradient (top) provided higher separation resolution than a 4–20% native polyacrylamide gel (bottom). Peaks 1–9 are (1) epidermal growth factor, (2) ovalbumin, (3) R-phycoerythrin subunit, (4) ovalbumin dimer, (5) β-galactosidase monomer subunit, (6) β-galactosidase dimer subunit, (7) R-phycoerythrin (intact heptamer), (8) β-galactosidase trimer subunit, and (9) β-galactosidase (intact tetramer). Figure from Ref. [27].

Streamlined analyses to assess protein microheterogeneity were also conducted using TGE by Linz and coworkers [141]. The protein ovalbumin (45 kDa) was added into a 30% F127 thermal gel, which was subsequently loaded throughout the entirety of a single-channel PDMS microfluidic device. Analyses conducted with the thermal gel in its low-viscosity state at 10 °C resulted in the continuous enrichment of

the protein as its band migrated along the channel. However, analyses conducted using solidified thermal gel at 30 °C exhibited unique behavior. The protein continued to enrich during the analysis while also separating into three distinct bands, which were attributed to different proteoform variants of the protein. Only with the 30% F127 thermal gel in its solid state could the variants be observed, which is unlike the 5% F127 coating required by Mikšík to observe protein microheterogeneity [151]. Interestingly, the Linz group observed that the separation was dynamically controllable with temperature [141]. A single enriched protein band at 10 °C could be separated into the three distinct variants by increasing the temperature to 30 °C during the analysis to solidify the gel (Figure 11A). Conversely, three separated bands at 30 °C could be recollected into a single band by decreasing the temperature to 10 °C during the analysis to liquify the gel (Figure 11B). This reversibility demonstrates the unique ability to control separations in thermal gels using temperature.

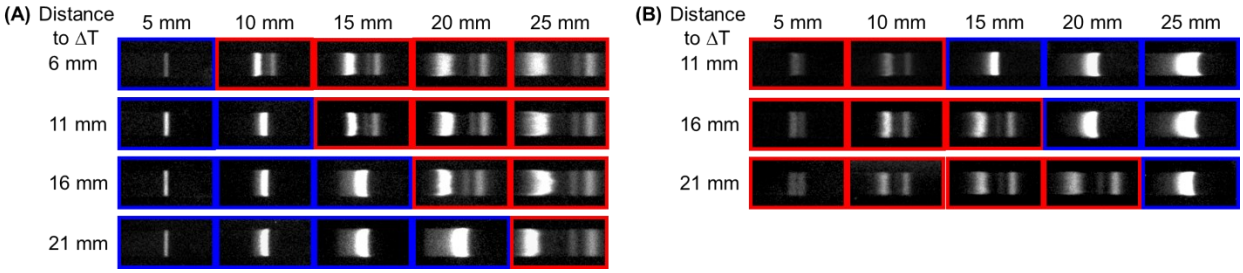

Figure 11. Single-channel microfluidic devices were loaded with 30% F127 containing 5 nM ovalbumin and analyzed by thermal gel electrophoresis. **(A)** A temperature step change from 10 °C (blue) to 30 °C (red) was applied to solidify the thermal gel when the band reached 6 mm, 11 mm, 16 mm, or 21 mm. **(B)** A temperature step change from 30 °C (red) to 10 °C (blue) was applied to liquify the thermal gel when the band reached 11 mm, 16 mm, or 21 mm. Altering the phase of the gel had an immediate impact on the separation by the next detection window. Figure from Ref. [141].

3.5 Voltage Response

Voltage is a tunable parameter in electrophoresis to alter migration time and separation efficiency. Diffusional band-broadening decreases with increasing voltage in CGE, but when voltages are too high, thermal band-broadening develops. Reducing conductivity is key to minimizing band-broadening due to increased thermal motion. The low dielectric constant of Pluronic allows for high voltages to be applied without creating excess Joule heating effects and band-broadening [100]. The conductivity in 20% F127 thermal gels is 50% that of a 20% polyacrylamide gel and 35% that of a 6% polyacrylamide gel [124], which enables higher voltages to be applied with Pluronic. A CGE study analyzing ssDNA obtained optimal performance in a 25% F127 thermal gel at 644 V/cm. Other studies applied high electric fields up to 1200

V/cm using 30% F127 thermal gel in microfluidic devices to separate dsDNA in <1 min without loss in resolution [100, 119, 133]. Under lower fields (515 V/cm and 387 V/cm), analysis times increased and separation resolution decreased [124]. However, resolution is dependent on both applied electric field and temperature. At 25 °C, the optimal resolution was achieved at 500 V/cm, but at 40 °C, the optimal resolution was achieved at 650 V/cm; however, the resolution was slightly higher at 25 °C and 500 V/cm [124].

Beyond measuring soluble biomolecules, thermal gels afford a medium for analyzing biological particles such as cells. Linz and coworkers developed an electrokinetic temperature control scheme using voltage to control channel temperature and, consequently, thermal gel viscosity [143]. Adjoining regions of liquid and solid thermal gel were created by applying higher voltage between certain microfluidic channels to selectively produce Joule heat in that region to regulate temperature. Cells readily migrated through the cooler liquid-phase gel and then accumulated against the warmed solid gel interface. The formation of the thermal gel barrier was reversible, as cells were able to freely flow within 1 s of removing the Joule heat from the cross channel. Although Joule heating is universally considered to be problematic in electrophoresis, Pluronic thermal gels attenuate conductivity, which prevents the formation of catastrophic air bubbles. This enables Joule heating to be harnessed to create reversible voltage-actuated barriers. Controlling thermal gel viscosity to enrich cells without the need for costly microfabricated heating elements simplifies analyses and reduces cost. This voltage-temperature control strategy has the potential to be employed for other bioanalytical measurements.

4. Concluding Remarks

Pluronic thermal gels possess interesting properties, which make them exciting materials for electrokinetic separations. The ability to control the viscosity and pore size of the gel through polymer concentration, buffer composition, and solvent enables the user to readily tailor conditions for their analytes of interest. The unique ability to dynamically use temperature to further control gel properties provides another dimension of flexibility for the analysis. In the simplest terms, the ease of use of Pluronic is unrivaled among gel matrices for GE. Capillaries and microfluidic devices can be easily filled with liquid-phase gel at lower temperatures before solidifying the gel at warmer temperatures. The temperature to achieve this can be readily tuned based on the specific Pluronic composition to accommodate analyses from below to above ambient conditions, which provides an extra degree of freedom to remain compatible with the target biological analytes. This temperature-dependent phase change also enables thermal gels to be replaced between analyses by cooling and rinsing to reuse devices. The user-friendly flexibility provided by Pluronic thermal gels are in stark contrast to the cumbersome traditional polymers (agarose, polyacrylamide) that cannot be adjusted after loading.

Despite the many benefits of thermal gels, their use in separations science has largely stagnated since peaking around the year 2000. Although it is unclear why, recent publications by our group and others have shown that we are only beginning to use thermal gels to their full potential. Standard polyacrylamide and agarose gels are static materials. Once cast, the gels cannot be altered to improve the analysis. With thermal gels, low-cost Peltiers can be readily integrated into a separations platform to control temperature and dynamically tune analysis conditions. The commercial availability of hobbyist electronic equipment provides an inexpensive means of regulating temperature—and thermal gel viscosity—that could not be easily achieved 20 years ago. Similarly, microfluidic devices can now be readily fabricated from inexpensive plastics to provide cost-effective analyses. Use of disposable microfluidic devices reduces total analysis times by eliminating the need to cool down CGE capillaries to remove and replace the gel before the next run. Improvements in electronic hardware and device manufacturing will help researchers entering the field as they work to fully take advantage of the dynamic polymer in numerous bioanalytical applications. Incorporating a dynamic element into the separation holds the potential to increase peak capacity and improve resolution similar to the use of gradients in liquid and gas chromatographies.

Thermal gels possess inherent benefits versus other polymers, but additional studies are still needed to fully define optimal biomolecule-specific operating conditions. For example, specific gel packing structures can be produced with rational foresight, which can maximize separation resolution. This requires systematic evaluations of micelle packing density and absolute viscosity to help guide operating conditions for bioanalyses. Fundamental studies on the length of PEO coronas and the width of the water-rich pores must be assessed in parallel to determine optimal entanglement with diverse analytes to enhance the separation. Studies are also needed to determine if the micelle cores can interact with analytes and whether they can be utilized to improve separation outcomes. A definitive separation mechanism of thermal gels still has not been established, but that information would provide further guidance to maximizing performance. The field would benefit from both experimental measurements and computational simulations to elucidate separative transport through thermal gels. The effects of electric field on the sieving mechanism is especially prudent.

Fundamental studies under isothermal conditions will provide insight to Pluronic thermal gels; however, the untapped potential of thermal gels lie in their dynamic nature. Changing the packing structure of the gel through time can maximize resolution for diverse biological analytes. Initial reports have begun to utilize the temperature-dependent nature of the polymer to alter analytical outcomes in the analysis of proteins and cells. These interesting demonstrations have characterized the tip of the iceberg, but more experiments are needed to explore the dynamic potential of thermal gels. Incorporating temperature gradients and/or voltage gradients within analyses have the potential to broaden the scope of utility, akin to improvements gained by using gradients in chromatography. Furthermore, methods must be developed to

analyze additional classes of biomolecules. Prior research has predominantly focused on DNA, but thermal gels have far greater potential to analyze a diverse assortment of soluble biomolecules as well as large biological particles such as cells and extracellular vesicles. Few reports have been published measuring these other biological analytes, which highlights the need for more work in these areas.

Although other analytical separations techniques such as HPLC and polyacrylamide gel electrophoresis (PAGE) have the advantage of a decades-long head-start, electrokinetic separations in thermal gel matrices offer potential benefits that cannot be achieved by the status quo. More research is needed to map out the parameter space and demonstrate new applications, but this presents exciting opportunities for inquisitive scientists interested in advancing the field of separations science. Given the universal need for separations in research science, manufacturing, and quality control applications, results from these endeavors will have a tangible impact on the real world.

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References

[1] J. Frenz, W.S. Hancock, High performance capillary electrophoresis, Trends Biotechnol., 9 (1991) 243-250.
[2] A.G. Ewing, R.A. Wallingford, T.M. Olefirowicz, Capillary electrophoresis, Anal. Chem., 61 (1989) 292A-303A.
[3] D. Wu, J. Qin, B. Lin, Electrophoretic separations on microfluidic chips, J. Chromatogr. A, 1184 (2008) 542-559.
[4] J.V. Pagaduan, V. Sahore, A.T. Woolley, Applications of microfluidics and microchip electrophoresis for potential clinical biomarker analysis, Anal. Bioanal. Chem., 407 (2015) 6911-6922.
[5] E.C. Rickard, M.M. Strohl, R.G. Nielsen, Correlation of electrophoretic mobilities from capillary electrophoresis with physicochemical properties of proteins and peptides, Anal. Biochem., 197 (1991) 197-207.
[6] A. Cifuentes, H. Poppe, Behavior of peptides in capillary electrophoresis: Effect of peptide charge, mass and structure, Electrophoresis, 18 (1997) 2362-2376.
[7] W.M. Sunada, H.W. Blanch, Polymeric separation media for capillary electrophoresis of nucleic acids, Electrophoresis, 18 (1997) 2243-2254.
[8] N.C. Stellwagen, E. Stellwagen, Effect of the matrix on DNA electrophoretic mobility, J. Chromatogr. A, 1216 (2009) 1917-1929.
[9] A. Chrambach, D. Rodbard, Polyacrylamide Gel Electrophoresis, Science, 172 (1971) 440-451.
[10] N.C. Stellwagen, Electrophoresis of DNA in agarose gels, polyacrylamide gels and in free solution, Electrophoresis, 30 (2009) S188-S195.
[11] D.L. Holmes, N.C. Stellwagen, The electric field dependence of DNA mobilities in agarose gels: A reinvestigation, Electrophoresis, 11 (1990) 5-15.
[12] N. Pernodet, M. Maaloum, B. Tinland, Pore size of agarose gels by atomic force microscopy, Electrophoresis, 18 (1997) 55-58.

[13] M.L. Greaser, C.M. Warren, Protein Electrophoresis in Agarose Gels for Separating High Molecular Weight Proteins, in: B.T. Kurien, R.H. Scofield (Eds.) Protein Electrophoresis: Methods and Protocols, Humana Press, Totowa, NJ, 2012, pp. 111-118.
[14] O. Vesterberg, A short history of electrophoretic methods, Electrophoresis, 14 (1993) 1243-1249.
[15] A.S. Cohen, B.L. Karger, High-performance sodium dodecyl sulfate polyacrylamide gel capillary electrophoresis of peptides and proteins, J. Chromatrogr. A, 397 (1987) 409-417.
[16] P.Y. Lee, J. Costumbrado, C.-Y. Hsu, Y.H. Kim, Agarose Gel Electrophoresis for the Separation of DNA Fragments, JoVE, (2012) e3923.
[17] A.V. Nichols, R.M. Krauss, T.A. Musliner, [24] Nondenaturing polyacrylamide gradient gel electrophoresis, Methods in Enzymology, Academic Press1986, pp. 417-431.
[18] J.J. Esposito, J.F. Obijeski, High Resolution Polyacrylamide Gradient Gel Electrophoresis, Prep. Biochem., 6 (1976) 431-442.
[19] J.-P. Doucet, B.J. Murphy, B.S. Tuana, Modification of a discontinuous and highly porous sodium dodecyl sulfate-polyacrylamide gel system for minigel electrophoresis, Anal. Biochem., 190 (1990) 209-211.
[20] A.E. Barron, H.W. Blanch, DNA Separations by Slab Gel, and Capillary Electrophoresis: Theory and Practice, Separation and Purification Methods, 24 (1995) 1-118.
[21] S.H. Sajjadi, E.K. Goharshadi, H. Ahmadzadeh, Heat dissipation in slab gel electrophoresis: The effect of embedded TiO2 nanoparticles on the thermal profiles, J. Chromatrogr. B, 1118-1119 (2019) 63-69.
[22] J.W. Jorgenson, K. Lukacs, Free-zone electrophoresis in glass capillaries, Clin. Chem., 27 (1981) 1551-1553.
[23] V. Miralles, A. Huerre, F. Malloggi, M.C. Jullien, A Review of Heating and Temperature Control in Microfluidic Systems: Techniques and Applications, Diagnostics, 3 (2013) 33-67.
[24] L. Bousse, S. Mouradian, A. Minalla, H. Yee, K. Williams, R. Dubrow, Protein Sizing on a Microchip, Anal. Chem., 73 (2001) 1207-1212.
[25] S. Yao, D.S. Anex, W.B. Caldwell, D.W. Arnold, K.B. Smith, P.G. Schultz, SDS capillary gel electrophoresis of proteins in microfabricated channels, Proc. Natl. Acad. Sci., 96 (1999) 5372-5377.
[26] Y. Li, J.S. Buch, F. Rosenberger, D.L. DeVoe, C.S. Lee, Integration of Isoelectric Focusing with Parallel Sodium Dodecyl Sulfate Gel Electrophoresis for Multidimensional Protein Separations in a Plastic Microfludic Network, Anal. Chem., 76 (2004) 742-748.
[27] S.H. Peli Thanthri, T.H. Linz, Controlling the separation of native proteins with temperature in thermal gel transient isotachophoresis, Anal. Bioanal. Chem., 415 (2023) 4163-4172.
[28] S.M. Kenyon, M.M. Meighan, M.A. Hayes, Recent developments in electrophoretic separations on microfluidic devices, Electrophoresis, 32 (2011) 482-493.
[29] C.J. Kristoff, L. Bwanali, L.M. Veltri, G.P. Gautam, P.K. Rutto, E.O. Newton, L.A. Holland, Challenging bioanalyses with capillary electrophoresis, Anal. Chem., 92 (2019) 49-66.
[30] B.C. Durney, C.L. Crihfield, L.A. Holland, Capillary electrophoresis applied to DNA: determining and harnessing sequence and structure to advance bioanalyses (2009–2014), Anal. Bioanal. Chem., 407 (2015) 6923-6938.
[31] L.J. Jin, B.C. Giordano, J.P. Landers, Dynamic Labeling during Capillary or Microchip Electrophoresis for Laser-Induced Fluorescence Detection of Protein−SDS Complexes without Pre- or Postcolumn Labeling, Anal. Chem., 73 (2001) 4994-4999.
[32] B.C. Giordano, L. Jin, A.J. Couch, J.P. Ferrance, J.P. Landers, Microchip Laser-Induced Fluorescence Detection of Proteins at Submicrogram per Milliliter Levels Mediated by Dynamic Labeling under Pseudonative Conditions, Anal. Chem., 76 (2004) 4705-4714.
[33] I. Mikšík, P. Sedláková, K. Mikulíková, A. Eckhardt, T. Cserhati, T. Horváth, Matrices for capillary gel electrophoresis—a brief overview of uncommon gels, Biomed. Chromatogr., 20 (2006) 458-465.
[34] B.A. Buchholz, E.A.S. Doherty, M.N. Albarghouthi, F.M. Bogdan, J.M. Zahn, A.E. Barron, Microchannel DNA Sequencing Matrices with a Thermally Controlled “Viscosity Switch”, Anal. Chem., 73 (2001) 157-164.

[35] F. Wan, W. He, J. Zhang, B. Chu, Reduced matrix viscosity in DNA sequencing by CE and microchip electrophoresis using a novel thermo-responsive copolymer, Electrophoresis, 30 (2009) 2488-2498.
[36] K. Nagase, T. Okano, Thermoresponsive-polymer-based materials for temperature-modulated bioanalysis and bioseparations, J. Mater. Chem. B, 4 (2016) 6381-6397.
[37] C. Yang, C. Chang, J. Wang, Q. Qu, X. Hu, Y. Wang, Thermally responsive polymer as a sieving matrix of proteins in capillary gel electrophoresis, Anal. Methods, 3 (2011) 2717-2720.
[38] B.P. Adrados, I.Y. Galaev, K. Nilsson, B. Mattiasson, Size exclusion behavior of hydroxypropylcellulose beads with temperature-dependent porosity, J. Chromatrogr. A, 930 (2001) 73-78.
[39] S.A. Archer-Hartmann, L.M. Sargent, D.T. Lowry, L.A. Holland, Microscale Exoglycosidase Processing and Lectin Capture of Glycans with Phospholipid Assisted Capillary Electrophoresis Separations, Anal. Chem., 83 (2011) 2740-2747.
[40] L. Bwanali, C.L. Crihfield, E.O. Newton, V.R. Zeger, S. Gattu, L.A. Holland, Quantification of the α2–6 Sialic Acid Linkage in Branched N-Glycan Structures with Capillary Nanogel Electrophoresis, Anal. Chem., 92 (2020) 1518-1524.
[41] C.L. Crihfield, L.A. Holland, Protein Sieving with Capillary Nanogel Electrophoresis, Anal. Chem., 93 (2021) 1537-1543.
[42] C.W. Kan, A.E. Barron, A DNA sieving matrix with thermally tunable mesh size, Electrophoresis, 24 (2003) 55-62.
[43] C.-W. Kan, E.A.S. Doherty, B.A. Buchholz, A.E. Barron, Thermoresponsive N,N-dialkylacrylamide copolymer blends as DNA sieving matrices with a thermally tunable mesh size, Electrophoresis, 25 (2004) 1007-1015.
[44] B.E. Root, M.L. Hammock, A.E. Barron, Thermoresponsive N-alkoxyalkylacrylamide polymers as a sieving matrix for high-resolution DNA separations on a microfluidic chip, Electrophoresis, 29 (2008) 4677-4683.
[45] B.C. Durney, J.A. Lounsbury, B.L. Poe, J.P. Landers, L.A. Holland, A thermally responsive phospholipid pseudogel: tunable DNA sieving with capillary electrophoresis, Anal. Chem., 85 14 (2013) 6617-6625.
[46] H. Musarurwa, N.T. Tavengwa, Stimuli-responsive polymers and their applications in separation science, React. Funct. Polym., 175 (2022) 105282.
[47] L.A. Holland, L.D. Casto-Boggess, Gels in Microscale Electrophoresis, Annu. Rev. Anal. Chem., 16 (2023) 161-179.
[48] I. Mikšík, Chapter 6 - Capillary Gel and Sieving Electrophoresis, in: C.F. Poole (Ed.) Capillary Electromigration Separation Methods, Elsevier (2018) pp. 143-165.
[49] P. Alexandridis, T. Alan Hatton, Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) block copolymer surfactants in aqueous solutions and at interfaces: thermodynamics, structure, dynamics, and modeling, Colloids Surf. A, 96 (1995) 1-46.
[50] A. Pitto-Barry, N. Barry, Pluronic® block-copolymers in medicine: From chemical and biological versatility to rationalisation and clinical advances, Polym. Chem., 5 (2014) 3291-3297.
[51] E.V. Batrakova, A.V. Kabanov, Pluronic block copolymers: Evolution of drug delivery concept from inert nanocarriers to biological response modifiers, J. Control. Release, 130 (2008) 98-106.
[52] J. Yu, H. Qiu, S. Yin, H. Wang, Y. Li, Polymeric Drug Delivery System Based on Pluronics for Cancer Treatment, Molecules, 2021.
[53] I. Jarak, C.L. Varela, E. Tavares da Silva, F.F.M. Roleira, F. Veiga, A. Figueiras, Pluronic-based nanovehicles: Recent advances in anticancer therapeutic applications, Eur. J. Med. Chem., 206 (2020) 112526.
[54] M.S.H. Akash, K. Rehman, S. Chen, Pluronic F127-Based Thermosensitive Gels for Delivery of Therapeutic Proteins and Peptides, Polym. Rev., 54 (2014) 573-597.
[55] R. Jaquilin P J, O.S. Oluwafemi, S. Thomas, A.O. Oyedeji, Recent advances in drug delivery nanocarriers incorporated in temperature-sensitive Pluronic F-127–A critical review, J. Drug Deliv. Sci. Technol., 72 (2022) 103390.

[56] C.R. Pacifico, L.G. Lundsted, Flake Form Nonionic Detergents, Soap Sanit. Chem., 26 (1950) 40-43, 73, 90.
[57] T.H. Vaughn, H.R. Suter, L.G. Lundsted, M.G. Kramer, Properties of Some Newly Developed Nonionic Detergents, J. Am. Oil Chem. Soc., 28 (1951) 294-299.
[58] J.C. Gilbert, J. Hadgraft, A. Bye, L.G. Brookes, Drug release from Pluronic F-127 gels, Int. J. Pharm., 32 (1986) 223-228.
[59] Safety Assessment of Poloxamers 101, 105, 108, 122, 123, 124, 181, 182, 183, 184, 185, 188, 212, 215, 217, 231, 234, 235, 237, 238, 282, 284, 288, 331, 333, 334, 335, 338, 401, 402, 403, and 407, Poloxamer 105 Benzoate, and Poloxamer 182 Dibenzoate as Used in Cosmetics, Int. J. Toxicol., 27 (2008) 93-128.
[60] A. Kabanov, J. Zhu, V. Alakhov, Pluronic Block Copolymers for Gene Delivery, Advances in Genetics, Academic Press (2005) pp. 231-261.
[61] P. Zarrintaj, J.D. Ramsey, A. Samadi, Z. Atoufi, M.K. Yazdi, M.R. Ganjali, L.M. Amirabad, E. Zangene, M. Farokhi, K. Formela, M.R. Saeb, M. Mozafari, S. Thomas, Poloxamer: A versatile tri-block copolymer for biomedical applications, Acta Biomater., 110 (2020) 37-67.
[62] M.G. Simões, A. Hugo, A. Gómez-Zavaglia, P.N. Simões, P. Alves, Formulation and Characterization of Stimuli-Responsive Lecithin-Based Liposome Complexes with Poly(acrylic acid)/Poly(N,N-dimethylaminoethyl methacrylate) and Pluronic Copolymers for Controlled Drug Delivery, Pharmaceutics, 14 (2022).
[63] A.V. Kabanov, E.V. Batrakova, V.Y. Alakhov, Pluronic® block copolymers as novel polymer therapeutics for drug and gene delivery, J. Control. Release, 82 (2002) 189-212.
[64] E. Batrakova, S. Lee, S. Li, A. Venne, V. Alakhov, A. Kabanov, Fundamental Relationships Between the Composition of Pluronic Block Copolymers and Their Hypersensitization Effect in MDR Cancer Cells, Pharm. Res., 16 (1999) 1373-1379.
[65] P. Alexandridis, J.F. Holzwarth, T.A. Hatton, Micellization of Poly(ethylene oxide)-Poly(propylene oxide)-Poly(ethylene oxide) Triblock Copolymers in Aqueous Solutions: Thermodynamics of Copolymer Association, Macromolecules, 27 (1994) 2414-2425.
[66] M. Khimani, U. Rao, P. Bahadur, P. Bahadur, Calorimetric and Scattering Studies on Micellization of Pluronics in Aqueous Solutions: Effect of the Size of Hydrophilic PEO End Blocks, Temperature, and Added Salt, J. Disper. Sci. Technol., 35 (2014) 1599-1610.
[67] R. Nagarajan, Solubilization of hydrocarbons and resulting aggregate shape transitions in aqueous solutions of Pluronic® (PEO–PPO–PEO) block copolymers, Colloids Surf. B, 16 (1999) 55-72.
[68] K. Mortensen, W. Brown, Poly(ethylene oxide)-Poly(propylene oxide)-Poly(ethylene oxide) Triblock Copolymers in Aqueous Solution. The Influence of Relative Block Size, Macromolecules, 26 (1993) 4128-4135.
[69] Z. Zhou, B. Chu, Anomalous micellization behavior and composition heterogeneity of a triblock ABA copolymer of (A) ethylene oxide and (B) propylene oxide in aqueous solution, Macromolecules, 21 (1988) 2548-2554.
[70] F. Artzner, S. Geiger, A. Olivier, C. Allais, S. Finet, F. Agnely, Interactions between Poloxamers in Aqueous Solutions: Micellization and Gelation Studied by Differential Scanning Calorimetry, Small Angle X-ray Scattering, and Rheology, Langmuir, 23 (2007) 5085-5092.
[71] H.J. Park, G.M. Treich, Z.D. Helming, J.E. Morgan, C.Y. Ryu, H.S. Hwang, G.Y. Jung, Micellar packing of pluronic block copolymer solutions: Polymeric impurity effects, Macromol. Res., 23 (2015) 13-20.
[72] C. Wu, T. Liu, B. Chu, D.K. Schneider, V. Graziano, Characterization of the PEO-PPO-PEO Triblock Copolymer and Its Application as a Separation Medium in Capillary Electrophoresis, Macromolecules, 30 (1997) 4575-4583.
[73] G. Wanka, H. Hoffmann, W. Ulbricht, Phase Diagrams and Aggregation Behavior of Poly(oxyethylene)-Poly(oxypropylene)-Poly(oxyethylene) Triblock Copolymers in Aqueous Solutions, Macromolecules, 27 (1994) 4145-4159.

[74] Z. Zhou, B. Chu, Light-Scattering Study on the Association Behavior of Triblock Polymers of Ethylene Oxide and Propylene Oxide in Aqueous Solution, J. Colloid Interface Sci., 126 (1988) 171-180.
[75] N.-J. Deng, Y.-Z. Luo, S. Tanodekaew, N. Bingham, D. Attwood, C. Booth, Gelation of micellar solutions of diblock-copoly (oxyethylene/oxybutylene) in aqueous K2SO4. An investigation of excluded volume effects, J Polym. Sci. Pol. Phys., 33 (1995) 1085-1096.
[76] W. Batsberg, S. Ndoni, C. Trandum, S. Hvidt, Effects of Poloxamer Inhomogeneities on Micellization in Water, Macromolecules, 37 (2004) 2965-2971.
[77] E. Kushan, E. Senses, Thermoresponsive and Injectable Composite Hydrogels of Cellulose Nanocrystals and Pluronic F127, ACS Appl. Bio Mater., 4 (2021) 3507-3517.
[78] C. Wu, T. Liu, B. Chu, Viscosity-Adjustable Block Copolymer for DNA Separation by Capillary Electrophoresis, Electrophoresis, 19 (1998) 231-241.
[79] M. Jalaal, G. Cottrell, N. Balmforth, B. Stoeber, On the rheology of Pluronic F127 aqueous solutions, J. Rheol., 61 (2017) 139-146.
[80] R.K. Prud'homme, G. Wu, D.K. Schneider, Structure and Rheology Studies of Poly(oxyethylene−oxypropylene−oxyethylene) Aqueous Solution, Langmuir, 12 (1996) 4651-4659.
[81] S. Costanzo, A.D. Sarno, M. D'Apuzzo, P.R. Avallone, E. Raccone, A. Bellissimo, F. Auriemma, N. Grizzuti, R. Pasquino, Rheology and morphology of Pluronic F68 in water, Phys. Fluids, 33 (2021) 043113.
[82] J.J. Escobar-Chavez, M. Lopez-Cervantes, A. Naik, Y.N. Kalia, D. Quintanar-Guerrero, A. Ganem-Quintanar, Applications of thermo-reversible pluronic F-127 gels in pharmaceutical formulations, J. Pharm. Pharm. Sci., 9 (2006) 339-358.
[83] R.L. Rill, Y. Liu, D.H. Van Winkle, B.R. Locke, Pluronic copolymer liquid crystals: unique, replaceable media for capillary gel electrophoresis, J. Chromatogr. A, 817 (1998) 287-295.
[84] R.L. Rill, B.R. Locke, Y. Liu, D.H.V. Winkle, Electrophoresis in Lyotropic Polymer Liquid Crystals, P. Natl. Acad. Sci. USA, 95 (1998) 1534-1539.
[85] F. Wan, J. Zhang, A. Lau, S. Tan, C. Burger, B. Chu, Nanostructured copolymer gels for dsDNA separation by CE, Electrophoresis, 29 (2008) 4704-4713.
[86] S. You, L. Wei, S. Shanbhag, D.H. Van Winkle, Nonmonotonic DNA-length-dependent mobility in pluronic gels, Phys. Rev. E, 95 (2017) 042602.
[87] Y. Kadam, R. Ganguly, M. Kumbhakar, V.K. Aswal, P.A. Hassan, P. Bahadur, Time Dependent Sphere-to-Rod Growth of the Pluronic Micelles: Investigating the Role of Core and Corona Solvation in Determining the Micellar Growth Rate, J. Phys. Chem. B, 113 (2009) 16296-16302.
[88] M. Vadnere, G. Amidon, S. Lindenbaum, J.L. Haslam, Thermodynamic studies on the gel-sol transition of some pluronic polyols, Int. J. Pharm., 22 (1984) 207-218.
[89] E. Hecht, H. Hoffmann, Kinetic and calorimetric investigations on micelle formation of block copolymers of the poloxamer type, Colloids Surf. A, 96 (1995) 181-197.
[90] P. Bahadur, P. Li, M. Almgren, W. Brown, Effect of potassium fluoride on the micellar behavior of Pluronic F-68 in aqueous solution, Langmuir, 8 (1992) 1903-1907.
[91] P.F. Wagler, U. Tangen, T. Maeke, J.S. McCaskill, Field programmable chemistry: Integrated chemical and electronic processing of informational molecules towards electronic chemical cells, Biosystems, 109 (2012) 2-17.
[92] M. Almgren, J. Van Stam, C. Lindblad, P. Li, P. Stilbs, P. Bahadur, Aggregation of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers in the presence of sodium dodecyl sulfate in aqueous solution, J. Phys. Chem., 95 (1991) 5677-5684.
[93] E. Hecht, H. Hoffmann, Interaction of ABA block copolymers with ionic surfactants in aqueous solution, Langmuir, 10 (1994) 86-91.
[94] E. Hecht, K. Mortensen, M. Gradzielski, H. Hoffmann, Interaction of ABA Block Copolymers with ionic Surfactants: Influence on Micellization and Gelation, The Journal of Physical Chemistry, 99 (1995) 4866-4874.
[95] J.-h. Ma, C. Guo, Y.-l. Tang, L. Chen, P. Bahadur, H.-z. Liu, Interaction of Urea with Pluronic Block Copolymers by 1H NMR Spectroscopy, J. Phys. Chem. B, 111 (2007) 5155-5161.

[96] K.-W. Kwon, M.J. Park, J. Hwang, K. Char, Effects of Alcohol Addition on Gelation in Aqueous Solution of Poly(ethylene oxide)-Poly(propylene oxide)-Poly(ethylene oxide) Triblock Copolymer, Polym. J., 33 (2001) 404-410.
[97] J. Armstrong, B. Chowdhry, J. Mitchell, A. Beezer, S. Leharne, Effect of Cosolvents and Cosolutes upon Aggregation Transitions in Aqueous Solutions of the Poloxamer F87 (Poloxamer P237): A High Sensitivity Differential Scanning Calorimetry Study, J. Phys. Chem., 100 (1996) 1738-1745.
[98] P. Parekh, K. Singh, D.G. Marangoni, P. Bahadur, Effect of alcohols on aqueous micellar solutions of PEO–PPO–PEO copolymers: A dynamic light scattering and 1H NMR study, J. Mol. Liq., 165 (2012) 49-54.
[99] P. Holmqvist, P. Alexandridis, B. Lindman, Modification of the Microstructure in Block Copolymer−Water−"Oil" Systems by Varying the Copolymer Composition and the "Oil" Type: Small-Angle X-ray Scattering and Deuterium-NMR Investigation, J. Phys. Chem. B, 102 (1998) 1149-1158.
[100] J. Zhang, M. Gassmann, W. He, F. Wan, B. Chu, Reversible thermo-responsive sieving matrix for oligonucleotide separation, Lab Chip, 6 (2006) 526-533.
[101] M. Zhang, M. Djabourov, C. Bourgaux, K. Bouchemal, Nanostructured fluids from pluronic® mixtures, Int. J. Pharm., 454 (2013) 599-610.
[102] K.K. Zeming, N.V. Thakor, Y. Zhang, C.-H. Chen, Real-time modulated nanoparticle separation with an ultra-large dynamic range, Lab Chip, 16 (2016) 75-85.
[103] A. Gencoglu, F. Camacho-Alanis, V.T. Nguyen, A. Nakano, A. Ros, A.R. Minerick, Quantification of pH gradients and implications in insulator-based dielectrophoresis of biomolecules, Electrophoresis, 32 (2011) 2436-2447.
[104] A. Nakano, F. Camacho-Alanis, A. Ros, Insulator-based dielectrophoresis with β-galactosidase in nanostructured devices, Analyst, 140 (2015) 860-868.
[105] C.L. Ng, H.K. Lee, S.F.Y. Li, Prevention of protein adsorption on surfaces by polyethylene oxide-polypropylene oxide-polyethylene oxide triblock copolymers in capillary electrophoresis, J. Chromatrogr. A, 659 (1994) 427-434.
[106] I. Mikšík, J. Charvátová, A. Eckhardt, Z. Deyl, Peptide mapping by capillary electrophoresis with Pluronic F127, J. Chromatogr. B, 800 (2004) 155-160.
[107] W. Hellmich, J. Regtmeier, T.T. Duong, R. Ros, D. Anselmetti, A. Ros, Poly(oxyethylene) Based Surface Coatings for Poly(dimethylsiloxane) Microchannels, Langmuir, 21 (2005) 7551-7557.
[108] S. Li, H.A. Stone, C.T. Murphy, A microfluidic device and automatic counting system for the study of C. elegans reproductive aging, Lab Chip, 15 (2015) 524-531.
[109] N. Aboud, D. Ferraro, M. Taverna, S. Descroix, C. Smadja, N. Thuy Tran, Dyneon THV, a fluorinated thermoplastic as a novel material for microchip capillary electrophoresis, Analyst, 141 (2016) 5776-5783.
[110] N.G. Batz, J.S. Mellors, J.P. Alarie, J.M. Ramsey, Chemical Vapor Deposition of Aminopropyl Silanes in Microfluidic Channels for Highly Efficient Microchip Capillary Electrophoresis-Electrospray Ionization-Mass Spectrometry, Anal. Chem., 86 (2014) 3493-3500.
[111] M. Cretich, M. Chiari, G. Pirri, A. Crippa, Electroosmotic flow suppression in capillary electrophoresis: Chemisorption of trimethoxy silane-modified polydimethylacrylamide, Electrophoresis, 26 (2005) 1913-1919.
[112] K. Srinivasan, C. Pohl, N. Avdalovic, Cross-Linked Polymer Coatings for Capillary Electrophoresis and Application to Analysis of Basic Proteins, Acidic Proteins, and Inorganic Ions, Anal. Chem., 69 (1997) 2798-2805.
[113] I. Mikšík, Z. Deyl, V. Kašicka, Capillary electrophoretic separation of proteins and peptides using Pluronic liquid crystals and surface-modified capillaries, J. Chromatrogr. B, 741 (2000) 37-42.
[114] K. Perez-Toralla, J. Champ, M.R. Mohamadi, O. Braun, L. Malaquin, J.L. Viovy, S. Descroix, New non-covalent strategies for stable surface treatment of thermoplastic chips, Lab Chip, 13 (2013) 4409-4418.
[115] A.P. Sudarsan, V.M. Ugaz, Printed Circuit Technology for Fabrication of Plastic-Based Microfluidic Devices, Anal. Chem., 76 (2004) 3229-3235.

[116] L. Martin, N. Damaso, D. Mills, Detection of single nucleotide polymorphisms (SNP) in equine coat color genes using SNaPshotTM multiplex kit or pluronic F-108 tri-block copolymer and capillary electrophoresis, Electrophoresis, 37 (2016) 2862-2866.
[117] R. Svingen, B. Akerman, Mechanism of Electrophoretic Migration of DNA in the Cubic Phase of Pluronic F127 and Water, J. Phys. Chem. B, 108 (2004) 2735-2743.
[118] S. You, D.H. Van Winkle, Single Molecule Observation of DNA Electrophoresis in Pluronic F127, J. Phys. Chem. B, 114 (2010) 4171-4177.
[119] J.B. Burton, C.L. Ward, D.M. Klemet, T.H. Linz, Incorporation of thermal gels for facile microfluidic transient isotachophoresis, Anal. Methods, 11 (2019) 4733-4740.
[120] J. Berka, Y.F. Pariat, O. Müller, K. Hebenbrock, D.N. Heiger, F. Foret, B.L. Karger, Sequence dependent migration behavior of double-stranded DNA in capillary electrophoresis, Electrophoresis, 16 (1995) 377-388.
[121] N.C. Stellwagen, Anomalous electrophoresis of deoxyribonucleic acid restriction fragments on polyacrylamide gels, Biochem., 22 (1983) 6186-6193.
[122] J. Zhang, D. Liang, W. He, F. Wan, Q. Ying, B. Chu, Fast separation of single-stranded oligonucleotides by capillary electrophoresis using OliGreen® as fluorescence inducing agent, Electrophoresis, 26 (2005) 4449-4455.
[123] C. Wu, T. Liu, B. Chu, A new separation medium for DNA capillary electrophoresis: self-assembly behavior of Pluronic polyol E99P69E99 in 1X TBE buffer, J. Non-Cryst. Solids, 235-237 (1998) 605-611.
[124] Y. Liu, B.R. Locke, D.H. Van Winkle, R.L. Rill, Optimizing Capillary Gel Electrophoretic Separations of Oligonucleotides in Liquid Crystalline Pluronic F127, J. Chromatrogr. A, 817 (1998) 367-375.
[125] S.J. Kim, G.W. Shin, S.J. Choi, H.S. Hwang, G.Y. Jung, T.S. Seo, Triblock copolymer matrix-based capillary electrophoretic microdevice for high-resolution multiplex pathogen detection, Electrophoresis, 31 (2010) 1108-1115.
[126] H.S. Hwang, G.W. Shin, H.J. Park, C.Y. Ryu, G.Y. Jung, Micellar ordered structure effects on high-resolution CE-SSCP using Pluronic triblock copolymer blends, Electrophoresis, 34 (2013) 518-523.
[127] G.W. Shin, H.S. Hwang, M.-H. Oh, J. Doh, G.Y. Jung, Simultaneous quantitative detection of 12 pathogens using high-resolution CE-SSCP, Electrophoresis, 31 (2010) 2405-2410.
[128] G.W. Shin, H.S. Hwang, S.W. Seo, M.-H. Oh, C.Y. Ryu, C.J. Salvo, S. Feldman, J. Doh, G.Y. Jung, A novel pathogen detection system based on high-resolution CE-SSCP using a triblock copolymer matrix, J. Sep. Sci., 33 (2010) 1639-1643.
[129] N. Damaso, L. Martin, P. Kushwaha, D. Mills, F-108 polymer and capillary electrophoresis easily resolves complex environmental DNA mixtures and SNPs, Electrophoresis, 35 (2014) 3208-3211.
[130] H.S. Hwang, G.W. Shin, A. Cohen, C.Y. Ryu, G.Y. Jung, Sieving properties of end group-halogenated Pluronic polymer matrix in DNA separation under nondenaturing CE analysis, Electrophoresis, 35 (2014) 2946-2950.
[131] J. Zhang, M. Gassmann, X. Chen, C. Burger, L. Rong, Q. Ying, B. Chu, Characterization of a Reversible Thermoresponsive Gel and Its Application to Oligonucleotide Separation, Macromolecules, 40 (2007) 5537-5544.
[132] R.L. Rill, Y. Liu, B.A. Ramey, D.H. Van Winkle, B.R. Locke, Capillary gel electrophoresis of nucleic acids in pluronic F127 copolymer liquid crystals, Chromatographia, 49 (1999) S65-S71.
[133] R.B. Schoch, M. Ronaghi, J.G. Santiago, Rapid and selective extraction, isolation, preconcentration, and quantitation of small RNAs from cell lysate using on-chip isotachophoresis, Lab Chip, 9 (2009) 2145-2152.
[134] D. Kuroda, Y. Zhang, J. Wang, N. Kaji, M. Tokeshi, Y.J.A. Baba, B. Chemistry, A viscosity-tunable polymer for DNA separation by microchip electrophoresis, Anal. Bioanal. Chem., 391 (2008) 2543-2549.
[135] V.M. Ugaz, R. Lin, N. Srivastava, D.T. Burke, M.A. Burns, A versatile microfabricated platform for electrophoresis of double- and single-stranded DNA, Electrophoresis, 24 (2003) 151-157.

[136] J.D. Epperson, J. Dodge, R.L. Rill, N.L. Greenbaum, Analysis of oligonucleotides and unincorporated nucleotides from in vitro transcription by capillary electrophoresis in Pluronic F127 gels, Electrophoresis, 22 (2001) 771-778.
[137] M.A. Cornejo, T.H. Linz, Multiplexed miRNA Quantitation Using Injectionless Microfluidic Thermal Gel Electrophoresis, Anal. Chem., 94 (2022) 5674-5681.
[138] M.A. Cornejo, T.H. Linz, Selective miRNA Quantitation with High-Temperature Thermal Gel Electrophoresis, Anal. Chim. Acta, In press (2023).
[139] R.L. Rill, M.A. Al-Sayah, Peptide separations by slab gel electrophoresis in Pluronic F127 polymer liquid crystals, Electrophoresis, 25 (2004) 1249-1254.
[140] P. Sedlakova, J. Svobodova, I. Miksik, Capillary electrophoresis of peptides and proteins with plug of Pluronic gel, J. Chromatrogr. B, 839 (2006) 112-117.
[141] S.H. Peli Thanthri, C.L. Ward, M.A. Cornejo, T.H. Linz, Simultaneous Preconcentration and Separation of Native Protein Variants Using Thermal Gel Electrophoresis, Anal. Chem., 92 (2020) 6741-6747.
[142] C.L. Ward, T.H. Linz, Characterizing the impact of thermal gels on isotachophoresis in microfluidic devices, Electrophoresis, 41 (2020) 691-696.
[143] M.A. Cornejo, T.H. Linz, Harnessing Joule heating in microfluidic thermal gel electrophoresis to create reversible barriers for cell enrichment, Electrophoresis, 42 (2021) 1238-1246.
[144] S. Gaisford, A.E. Beezer, J.C. Mitchell, Diode-Array UV Spectrometric Evidence for Cooperative Interactions in Binary Mixtures of Pluronics F77, F87, and F127, Langmuir, 13 (1997) 2606-2607.
[145] M. Chung, D. Kim, A.E. Herr, Polymer sieving matrices in microanalytical electrophoresis, Analyst, 139 (2014) 5635-5654.
[146] A. Guttman, Capillary sodium dodecyl sulfate-gel electrophoresis of proteins, Electrophoresis, 17 (1996) 1333-1341.
[147] C. Heller, Capillary electrophoresis of proteins and nucleic acids in gels and entangled polymer solutions, J. Chromatrogr. A, 698 (1995) 19-31.
[148] H. Xu, C.J. Minter, S. Nagasaka, T. Ito, D.A. Higgins, Elongation, Alignment, and Guided Electrophoretic Migration of ds-DNA in Flow-Aligned Hexagonal F127 Gels, J. Phys. Chem. B, 118 (2014) 4151-4159.
[149] I. Mikšík, Z. Deyl, Application of Pluronic copolymer liquid crystals for the capillary electrophoretic separation of collagen type I cyanogen bromide fragments, J. Chromatrogr. B, 739 (2000) 109-116.
[150] T. Krizek, P. Coufal, E. Tesarova, J. Sobotnikova, Z. Bosakova, Pluronic F-127 as the buffer additive in capillary entangled polymer electrophoresis: some fundamental aspects, J. Sep. Sci., 33 (2010) 2458-2464.
[151] I. Mikšík, A. Eckhardt, E. Forgács, T. Cserháti, Z. Deyl, The effect of sodium dodecyl sulfate and Pluronic F127 on the electrophoretic separation of protein and polypeptide test mixtures at acid pH, Electrophoresis, 23 (2002) 1882-1886.