BIOMEDICAL CHROMATOGRAPHY
Biomed. Chromatogr. **20**: 458–465 (2006)
Published online in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/bmc.640

REVIEW

Matrices for capillary gel electrophoresis—a brief overview of uncommon gels

Ivan Mikšík,[1,2]* Pavla Sedláková,[1] Kateřina Mikulíková,[1] Adam Eckhardt,[1,2] Tibor Cserhati[3] and Tibor Horváth[3]

[1]Institute of Physiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
[2]Cardiovascular Research Centre, Prague, Czech Republic
[3]Institute of Materials and Environmental Chemistry, Chemical Research Center of the Hungarian Academy of Sciences, Budapest, Hungary

Received 3 November 2005; accepted 10 January 2006

ABSTRACT: This article gives an overview of uncommon replaceable matrices (gels) for capillary gel electrophoresis. This electrophoretic technique is useful mainly for the separation and analysis of biopolymers—nucleic acids and their fragments, and proteins/peptides. Commonly used gels are not reviewed. Those mentioned and discussed here are gels containing saccharides, newly developed acrylamide-based gels and thermoadjustable viscosity polymers, namely triblock copolymers and grafted polyacrylamide. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: capillary gel electrophoresis; gels; separation matrices; triblock copolymers

INTRODUCTION

Capillary gel electrophoresis exploiting gel matrices as separation media is a powerful technique for the separation of biopolymers, such as proteins and DNA fragments, with the advantages of rapidity, high resolving power and minute amounts of samples required (Hsieh *et al.*, 2006; Kennedy *et al.*, 1999). A small-size biopolymer easily migrates through the pores of the polymer solution (sieving matrix) and is detected earlier in the anode than a large one. The most commonly prepared gel solutions are linear polymers such as linear polyacrylamide (LPA), poly(dimethylacrylamide) (PDMA), poly(ethylene oxide) (PEO), cellulose derivatives such as hydroxyethyl cellulose (HEC) and hydroxypropylmethyl cellulose (HPMC), and poly(vinylpyrrolidone) (PVP), which possess the advantages of a low fluorescence background, low viscosity, self-coating properties and a high sieving ability. When compared with cross-linked gels, polymer solutions have the advantages of easy preparation, low viscosity and flexibility (Sartori *et al.*, 2003). Because of its low viscosity, filling small separation channels with polymer solution is not problematic, which allows replacement of the polymer solution after each run. As a result, the separation channels can be used for many runs (e.g. >100 runs), reducing the cost of analysis. By comparison, generally only the first few runs provide acceptable resolution and reproducibility when using gels that are prepared inside the separation channel (Cifuentes *et al.*, 1993). Many reviews of gels for capillary gel electrophoresis exist (Barbier and Viovy, 2003; Hsieh *et al.*, 2005; Chu and Liang, 2002). Many theoretical models of the sieving mechanisms during the separation of macromolecules are described there. For details concerning these models we suggest reading the detailed review by Sartori *et al.* (2003).

In the analysis of proteins, surfactants such as sodium dodecyl sulfate (SDS) are frequently added to denature proteins, enables the separation of proteins according to their molecular masses (M_r). Using the linear relationship between the mobilities of known proteins or DNA molecules and their M_r, the M_r values of unknown proteins or DNA molecules can be obtained

*Correspondence to: I. Mikšík, Institute of Physiology, Academy of Sciences of the Czech Republic, Videnska 1083, 14220 Praha 4, Czech Republic.
E-mail: miksik@biomed.cas.cz

Abbreviations used: BEB, polyoxybutylene-polyoxyethylene-polyoxybutylene; CMC, critical micellar concentration; DEA, N,N′-diethylacrylamide; DMA, N,N′-dimethylacrylamide; EOF, electroosmotic flow; HEC, hydroxyethyl cellulose; HPMC, hydroxypropylmethyl cellulose; IPN, interpenetrating networks; LPA, linear polyacrylamide; Mr, molecular masses; N,N-dimethylacrylamide (DMA); NEEA, N-ethoxyethylacrylamide; NMEA, N-methoxyethylacrylamide; PDMA, poly(dimethylacrylamide); PDMA, poly(N,N-dimethylacrylamide); PEG, polyethylene glycol; PEO, poly(ethylene oxide); PHEA, poly-N-hydroxyethylacrylamide; PNIPAM-g-PEO, poly(N-isopropylacrylamide)-g-poly(ethyleneoxide); PVP, poly(vinylpyrrolidone).

Contract/grant sponsor: Czech–Hungarian cooperation programme.
Contract/grant sponsor: Grant Agency of the Czech Republic; Contract/grant number: 203/03/0716, 203/05/2539.
Contract/grant sponsor: Center for Heart Research; Contract/grant number: 1M6798582302.
Contract/grant sponsor: Research Project AV0Z50110509.

(Banks, 1998; Gilar *et al.*, 1998; Guttman *et al.*, 2003; Zhu and Feng, 2005).

The term capillary gel electrophoresis overlaps in some aspects with capillary electrochromatography in monolithic columns (for a review see Svec *et al.*, 2003). From a historical point of view, Hjertén *et al.* (1989) introduced the term 'continuous polymer bed' for compressed polyacrylamide gel; subsequently many other terms were used (e.g. continuous column support) and nowadays the well-established term is 'monolith' (Svec and Tennikova, 2003). The expression 'monolithic' related to rigid macroporous polymers prepared by bulk polymerization in a closed mold (Viklund *et al.*, 1996; in the case of capillary electrochromatography this polymerization occurs inside the capillary).

There are a plenty of reviews devoted to the various areas of application of capillary electromigration techniques and also covering capillary gel electrophoresis: their application in the clinical laboratory (Petersen *et al.*, 2003), forensic drug analysis (Anastos *et al.*, 2005), detection of drug–protein adducts (Zhou, 2003), synthetic polymers (Cottet *et al.*, 2005), food analysis (Garcia-Canas *et al.*, 2004), multidimensional separation of peptides (Issaq *et al.*, 2005), analysis of colloidal/nano-particles including microorganisms (Rodriguez and Armstrong, 2004), haplotyping techniques (DNA fragment-based analysis; Szantai *et al.*, 2005) or the use of ionic polymers for the separation of ions (Fritz *et al.*, 2002).

There are two reasons for restricting this review to only uncommon linear (replaceable) gels/polymers:

(1) the overlap in the terms capillary electrochromatography in monolithic columns and capillary gel electrophoresis (as mentioned above);
(2) capillary gel electrophoretic methods are routinely used for the analysis of nucleic acids (e.g. PCR products) and proteins according to the size of the fragments/molecular weight.

For these reasons the review presented here is restricted to capillary electrophoresis exploiting non-traditional, uncommon replaceable gels.

It is not simple to devise a classification scheme for the matrices/gels used in capillary gel electrophoresis. The vast majority of advanced gels are copolymers (but not all of them). The following overview is subdivided according to chemical or typical physical/separation properties.

GELS CONTAINING SACCHARIDES

The use of cellulose-derived polysaccharides, such as hydroxyethylcellulose-derivative polymers, is often described for the separation of biopolymers, for example of viral double-stranded RNA fragments (Shambaugh *et al.*, 2004). Another type of polysaccharide, pullulan, was also found to be a useful matrix for the separation of proteins (Nakatani *et al.*, 1994, 1996). The following method was used for determining the molecular mass of proteins: the inner surface of the capillary was deactivated by coating it with linear polyacrylamide. The capillary was filled with a low-viscosity solution of pullulan (with a molecular mass range of 50,000–100,000) at a concentration of between 3 and 10% w/v of pullulan. A good linear relationship was obtained between the mobility and logarithm of the molecular mass of SDS–proteins. The separation obtained was in accordance with the Ogston theory.

It is worth mentioning that some other water-soluble, native polysaccharides (such as amylose, laminaran and pullulan) and derived polysaccharides (methyl cellulose, hydroxypropyl cellulose, and carboxymethyl amylose sodium salt, CM-Am) were also used as chiral selectors in capillary electrophoresis. Pullulan and amyloses provided the same migration order for the studied enantiomers; the migration order of the enantiomers for cellulose derivatives and laminaran as well as with β-cyclodextrin was opposite to that for amylose and pullulan (Chankvetadze *et al.*, 1997).

Chiari *et al.* (2001) prepared copolymers of acrylamide and allyl gluconic and lactobionic acid. These copolymers had a relative molecular mass of 288 and 180 kDa, respectively. The copolymers of acrylamide and allyl gluconic acid have a high sieving capacity for double-stranded DNA fragments and provide a performance similar to that of a solution of hydroxyethylcellulose (HEC) of comparable viscosity. This copolymer self-coats onto the capillary wall, allowing DNA fragments to be efficiently separated in an uncoated capillary.

It is also worth mentioning that cationic starch derivatives can be used as dynamic coating additives for protein analysis in capillary electrophoresis (Sakai-Kato *et al.*, 2006).

PARTICLES IN THE GEL

Gold nanoparticles can be used in the separation of double-stranded DNA by microchip capillary electrophoresis using poly(ethylene oxide) (Lin *et al.*, 2003). Microchannels on poly(ethylene methacrylate) were three-layer-coated in sequence with poly(vinyl pyrrolidone), poly(ethylene oxide) and 13 nm gold nanoparticles. The inner dimensions of the channels were 75×75 μm, and total length 5 cm (effective length 3 cm). The channels were filled with 1.5% poly(ethylene oxide) containing gold nanoparticles in a 100 mM glycine–citrate buffer at pH 9.2 and were suitable for the separation of DNA markers V and VI ranging in size from 8 to 2176 base pairs. In conjunction with

stepwise changes in the concentration of ethidium bromide in the buffer (0.5 and 5 mg/mL), this method provides improved resolution and sensitivity for DNA markers V and VI.

ACRYLAMIDE-BASED COPOLYMERS

Acrylamide is a common monomer for the preparation of polymers and copolymers serving as media for capillary gel electrophoresis. In this overview only new or untraditional copolymers will be mentioned. Because acrylamide is commonly used, copolymers containing this compound can be found in other sections of this paper, for example copolymers of acrylamide and allyl gluconic and lactobionic acid are described in the previous section covering gels containing saccharides, or in the next section on thermo-responsive polymers.

An amphiphilic acrylamide-based copolymer was prepared by micellar copolymerization of the hydrophilic monomer acrylamide and silicone-containing hydrophobic comonomer tris(trimethylsiloxy)-methacryloxypropylsilane. This copolymer associates with micelles in water. It was demonstrated that these matrices can be used as separation media in capillary electrophoresis for the separation of DNA (the molar ratio of acrylamide to hydrophobic silicone monomer being 20:1 and 100:1, respectively). Experimental results indicate that the copolymer with higher hydrophobe content showed zero separation efficiency while the lower one separated most DNA fragments clearly from a fX174/Hae III digest at very low copolymer concentrations of 0.1 wt% (Li *et al.*, 2004).

Comb-like copolymers with a polyacrylamide backbone and poly(*N*,*N*-dimethylacrylamide) grafts were also prepared (Barbier *et al.*, 2002). These copolymers are able to combine the superior sieving properties of polyacrylamide with the self-coating properties of polydimethylacrylamide. The authors studied the use of these gels for DNA sequencing and so examined structural parameters such as the grafting density and molecular mass of the polymer. Good performance appears to be achieved with a relatively large range of parameters. Excellent separation was achieved even with matrices that have a viscosity as low as 200 mPa/s.

Another interesting polymer material is poly-*N*-hydroxyethylacrylamide (its commercial name is polyDuramide). It is a hydrophilic, self-coating polymer that can be used as a matrix for DNA sequencing by capillary electrophoresis (Albarghouthi *et al.*, 2002). This replaceable polymer matrix, based on the monomer *N*-hydroxyethylacrylamide (HEA), has been synthesized for use in DNA separation by microchannel electrophoresis. The monomer is more hydrophilic than acrylamide and *N*,*N*-dimethylacrylamide. Polymers were synthesized by free radical polymerization in aqueous solution. Poly-*N*-hydroxyethylacrylamide (PHEA) exhibits good capillary-coating properties via adsorption from aqueous solution, and reduces electroosmotic flow (EOF), similar to poly-*N*,*N*-dimethylacrylamide. PHEA coatings are stable for over 600 h of electrophoresis. Capillary electrophoresis in a bare fused capillary with PHEA (6% w/v, molecular weight $M_r = 5.2 \times 10^6$ g/mol) can resolve over 620 bases of contiguous DNA sequence within 3 h. These results demonstrate the potential of PHEA matrices for high-throughput DNA analysis by microchannel electrophoresis.

PHEA was successfully used for coating the capillary in the analysis of *Escherichia coli* O157:H7 bacteria by a combination of immunofluorescent staining and capillary electrophoresis (Kourkine *et al.*, 2003).

INTERPENETRATING NETWORKS

Interpenetrating networks (IPN) constituting polymers with totally different chemical properties were successfully used as gel media for capillary electrophoresis. Song *et al.* (2001) developed highly entangled IPNs of polyacrylamide and polyvinylpyrrolidone (PVP). The incompatibility of these two polymers was suppressed by the polymerization of acrylamide in a matrix of PVP solution. These interpenetrating networks were suitable for the analysis of double-stranded DNA. It was possible to separate 22 fragments from pBR322/HaeIII DNA, including the doublet of 123/124 bp with 2% w/v PVP (weight-average molecular mass $M_r = 1 \times 10^6$ g/mol) + 1% w/v polyacrylamide ($M_r = 4 \times 10^5$ g/mol). A similar resolution was achieved by using polyacrylamide ($M_r = 4 \times 10^5$ g/mol) with concentrations higher than 6% w/v. However, PVP alone ($M_r = 1 \times 10^6$ g/mol) at a concentration as high as 15% w/v was unsuitable for this separation.

Another non-cross-linked interpenetrating polymer network suitable for the separation of double-stranded DNA fragments, consisting of two other polymers [poly(*N*,*N*-dimethylacrylamide, PDMA, and polyvinylpyrrolidone] was developed by Wang *et al.* (2002). It was prepared by polymerizing *N*,*N*-dimethylacrylamide (DMA) monomers directly in PVP (molecular weight $M_r = 1 \times 10^6$ g/mol) buffer solution. It was demonstrated that IPN had a much higher viscosity than the simple mixture containing the same amount of PDMA and PVP. At optimal conditions (4% w/v PDMA and 4% PVP w/v), the 22 fragments of pBR322/HaeIII DNA were successfully separated within 15 min, with a resolution better than 1.0 for 123/124 bp.

Wang *et al.* (2005) also developed a quasi-interpenetrating network formed by polyacrylamide and poly(*N*,*N*-dimethylacrylamide) for DNA sequencing

analysis. Quasi-IPN was able to achieve one-color DNA sequencing of up to 1000 bases in 39 min, or 1200 bases in 60 min when quasi-IPN yielded a read length of up to 700 bases of contiguous sequence (50–750 bases) in 35 min with 99.6% accuracy, or 750 bases of contiguous sequence (50–800 bases) in 37 min with 98.0% accuracy.

BLOCK COPOLYMERS

Block copolymers are an interesting category of polymers. These copolymers can self-assemble to form micelle structures in selective solvents (in capillary electrophoresis these are aqueous buffers). This property makes these materials highly interesting for capillary gel electrophoresis and they are another alternative to linear polymeric materials. Some interesting block copolymers have been developed, consisting of polyethylene glycol end-capped with fluorocarbon tails (Menchen *et al.*, 1996) or *n*-dodecane-poly(ethylene oxide)-*n*-dodecane (Magnusdottir *et al.*, 1998). At present, the most frequently used block copolymer is poly(ethylene oxide)–poly(propylene oxide)–poly (ethylene oxide) Pluronic F-127 (Rill and Al-Sayah, 2004).

Block copolymers consisting of polyethylene glycol (PEG) end-capped with fluorocarbon (C_6F_{2n+1}) tails form flower-like micelle structures in aqueous media when present above the CMC (critical micellar concentration), with the hydrophobic fluorocarbons forming the core and PEG dangling outside forming the hydrophilic corona (Menchen *et al.*, 1996). The micelles aggregate to form a network structure with increasing polymer concentration. This structure is bridged by PEG blocks and results in increasing viscosity. For this reason the copolymer can be used at low concentration (below 6%) for DNA separation. Optimum sequencing results were obtained from a 6% solution of a 1:1 mixture of C_6F_{13} end-capped and C_8F_{17} end-capped PEG 35,000 (i.e. molecular mass 35,000). The resolution limit of fluorescent-dye-labeled sequencing products in this formulation was 450 bases (Menchen *et al.*, 1996).

Another block (triblock) copolymer was *n*-dodecane–poly(ethylene oxide)–*n*-dodecane. Magnusdotirr *et al.* (1998) used it for the separation of oligonucleotides. The structure of micelles was similar to that of polyethylene glycol end-capped with fluorocarbonyl tails—at concentrations above 4% they formed a micellar network in which the dodecane micellar cores were bridged by polyoxyethylene segments. A good separation of Pd(A)25–30 and Pd(A)40–60 oligonucleotides was obtained at concentrations between 5.1 and 10.1% (Magnusdottir *et al.*, 1998). Block (triblock) copolymers can also serve as thermo-responsive gels.

THERMO-RESPONSIVE POLYMERS

The most interesting copolymers are thermoadjustable-viscosity polymers. A typical example of this category would be Pluronic polymers. Pluronic polymers are triblock uncharged copolymers with the general formula $[\text{poly(ethylene oxide)}]_x[\text{poly(propylene oxide)}]_y[\text{poly(ethylene oxide)}]_x$ (Pluronic is a registered trade name of BASF Performance Chemicals, Mount Olive, NJ, USA). Of these types of copolymers, Pluronic F127 (with coefficient values of roughly x = 100 and y = 70 and a molecular mass of about 13,000) is often used. These copolymers have the typical features of surfactants and self-associate into large micelles (Linse, 1993; Malmsten and Lindman, 1992; Mortensen *et al.*, 1992; Mortensen and Talmon, 1995; Wanka *et al.*, 1994). Self-association is favored by increasing concentration and temperature. The less polar poly(propylene oxide) chain segments are desolvated and segregate into a hydrophobic micelle core surrounded by a soft 'brush' of highly hydrated, flexible poly(ethylene oxide) chains. Pluronic copolymers form both isotropic and anisotropic liquid crystalline 'gels'. The type of phase (isotropic, cubic, hexagonal or lamellar) depends not only on the structural features of the polymer but also on its concentration and temperature. This means in practice that a Pluronic which is soluble at low temperature can gellify with a temperature increase, e.g. Pluronic F127 at a concentration of 20% is a freely flowing liquid at refrigerator temperature (5°C). At this stage the polymer can be easily introduced into the capillaries. At room temperature (20°C) this liquid forms gels (Rill *et al*., 1998a,b; Wu *et al*., 1997, 1998a,b). It was proposed that these copolymers, owing to their unique features, might represent useful media for electrophoretic separations of biological macromolecules (reviewed by Rill *et al.*, 1998a).

Double-stranded DNA (Liang and Chu, 1998; Rill *et al.*, 1998b; Wu *et al.*, 1997, 1998b) and oligonucleotides (Liu *et al.*, 1998; Rill *et al.*, 1998b) have been separated using Pluronic F127. Oligonucleotides can be successfully separated at 25% Pluronic F127 at 30°C or at 20% Pluronic F127 at 50°C (Liu *et al.*, 1998).

Pluronic media was also used for the separation of peptides when 7.5% Pluronic F127 (in a pH 2.5, 10 mM Tris and 75 mM phosphate buffer) was used for the separation of the CNBr peptides of collagen (Miksik and Deyl, 2000). The separation was significantly improved in comparison with the separation achieved in the buffer alone (without the Pluronic). The temperature used was 20°C; a higher temperature (50°C) influenced migration time but not resolution or migration order. The use of a higher concentration of gel caused too long a migration time and produced many 'bumps' on the baseline. Pluronic medium was validated as suitable media for the separation of peptides when it is

assumed that separation of proteins/peptides in the presence of Pluronic in the background electrolyte occurs on a charge/mass ratio basis with molecular sieving effects acting as a secondary partition mechanism (Miksik *et al.*, 2000). Pluronic F127 offers clear-cut separations of standard proteins up to a relative molecular mass of 5×10^4 Da and enables the observation of protein/polypeptide microheterogeneity where applicable (Miksik *et al.*, 2002). The benefits of Pluronic F127 for peptide mapping by capillary electrophoresis have also been demonstrated (Miksik *et al.*, 2004).

The usability of Pluronic F127 for slab gel electrophoresis of peptides has also been shown (Rill and Al-Sayah, 2004). Separations of myoglobin tryptic peptides were obtained by electrophoresis on slab gels of 24% Pluronic F127 or 15% polyacrylamide using the alkaline Laemmli buffer system (without SDS) and the results were comparable with both systems (this was verified using a two-dimensional system that coupled Pluronic and polyacrylamide gels).

Other interesting media with temperature-controlled viscosity are mixtures of triblock copolymers. Liu *et al.* (2001) developed a method for separating double-stranded DNA (dsDNA) fragments. A mixture of two polyoxybutylene-polyoxyethylene–polyoxybutylene (BEB) triblock copolymers ($B_6E_{46}B_6$ and $B_{10}E_{271}B_{10}$, respectively) served as gel media. The mixture of these two triblock copolymers forms mixed flower-like micelles in dilute solution and at higher polymer concentrations forms a homogeneous gel-like open-network with hydrophobic clusters as cross-linking points. As a polyoxyalkylene block copolymer gel, the separation medium has some specific advantages, including the temperature-dependent sol–gel transition that makes sample injection easy, and the self-coating of the inner capillary wall. The elution time was shorter and the separation resolutions improved, especially for small dsDNA fragments, when compared with poly(ethylene oxide)–poly(propylene oxide) poly(ethylene oxide)-type separation media, e.g. Pluronic F127. The base pair sequence was fully resolved for dsDNA fragments of over 100 base pairs (Liu *et al.*, 2001).

In another work (Liang *et al.*, 2001), the authors stated that the optimal concentration for this mixture of triblock copolymers is 3% (w/v) $B_{10}E_{270}B_{10}$ and 5% (w/v) $B_6E_{46}B_6$, as determined when considering both speed and resolution. A resolution of 1.3 was achieved on the separation of 123/124 base pairs from a pBR322/HaeIII digest within 20 min (Liang *et al.*, 2001). The resolution was highly sensitive to block length: polyoxybutylene blocks play a major role in the gel-forming process. It was stated that the separation results are better with a mixture of these triblock copolymers than with Pluronic F127, especially with small fragments. However, it is difficult to control reproducibility in the length of blocks, and mixture formulation has to be optimized each time to reach optimal resolution.

Another example of thermo-responsive polymers are grafted copolymers, e.g. poly(*N*-isopropylacrylamide)-g-poly(ethyleneoxide) (PNIPAM-g-PEO; Liang *et al.*, 1999a,b). The PNIPAM-g-PEO copolymer is a high-molecular polymer (M_r > 10 milion) with densely grafted PEO chains (one PEO chain per 30 repeating units of backbone chain and an average PEO chain length of about 22 segments). This grafted copolymer has good self-coating properties and its viscosity is slightly temperature-dependent (and so adjustable). At low concentrations and room temperatures the copolymer has a random coil conformation; at a temperature above 31°C, the copolymer begins to shrink and collapse. One base pair resolution was achieved using 8% w/v PNIPAM-g-PEO in 1 × TBE (Tris-borate-ethylenediaminetetraaceticacid) buffer. The PNIPAM-g-PEO solutions had a high sieving ability for relatively small-sized DNA fragments and also ΦX174/HaeIII digest could be clearly separated.

The copolymer PAM-g-PNIPAM consisting of a hydrosoluble backbone of PAM grafted with short-chain PNIPAM was described by Sudor *et al.* (2001). The thermothickening of micelle-like aggregates depends on temperature and buffer additives. The best copolymer for the separation of 100 bp (and DNA segments) was a long backbone ($1.5-2 \times 10^6$) of PAM grafted with a small fraction (less than 10%) of relatively short side chains (around 10,000) of PNIPAM. Without complete optimization, a resolution of order 0.5 could be achieved for segments around 800 bases differing by 1 base (Sudor *et al.*, 2001), corresponding to a very reasonable limit for read length with current base-calling software.

Buchholz *et al.* (2001) used the term 'switchable materials' for thermoresponsive polymer matrices that exhibit a reversible, temperature-controlled 'viscosity switch'. A series of linear copolymers of *N*,*N*′-dimethylacrylamide (DMA) and *N*,*N*′-diethylacrylamide (DEA) with different monomer compositions such as the copolymer consisting of 42% DEA and 58% DMA and that of 53% DEA and 47% DMA have been prepared and tested for DNA separations (Buchholz *et al.*, 2001, 2002). The authors pointed out that the resolution decreases with increasing hydrophobicity of the polymer solution (Albarghouthi *et al.*, 2001; Buchholz *et al.*, 2001, 2002; Kan *et al.*, 2003).

Another class of thermogelling polymer networks is based on poly-*N*-alkoxyalkylacrylamides. Polymers and copolymers of *N*-ethoxyethylacrylamide (NEEA) and *N*-methoxyethylacrylamide (NMEA) were synthesized by aqueous-phase free-radical polymerization. These copolymer matrices exhibit 're-entrant'-type volume phase transitions, forming entangled networks with

high shear viscosity at low (<20°C) and high (>35°C) temperatures, and undergoing a 'coil-to-globular', lower critical solution temperature (LCST)-like phase transition over an intermediate temperature range (20–35°C). The matrix viscosity is relatively low at room temperature (25°C), and increases rapidly above 35°C. The extent of the intermediate viscosity drop and the final viscosity increase depends on the composition of the copolymers. These polymer networks can serve as DNA sequencing matrices for high-throughput microchannel electrophoresis in capillary arrays. These gels provide enhanced resolution of both small and large DNA sequencing fragments and longer sequencing read lengths, in comparison to appropriate control (closely related, non-thermogelling) polymer networks. In particular, a copolymer comprising 90% w/w NMEA and 10% w/w NEEA, with a molecular mass of ~2 MDa, delivers a 600 base read at 98.5% base-calling accuracy in 100 min of electrophoresis (Kan *et al.*, 2003).

Dynamic polymers of surfactant molecules can be also classified as thermo-responsive gels. These polymers are composed of a hydrophilic polyoxyethylene oligomer (E_8) with a hydrophobic C_{16} alkyl chain grafted onto one end. This matrix is an amphiphilic macromolecule and forms worm-like micelles. The size of micelle is temperature dependent—it increases with increasing temperature. The matrix can be used for the separation of DNA when the micelles entangle with each other. Resolution is poor at low temperatures (the micelles are not entangled) and improves with increasing temperature (when the micelles entangle). At temperatures above the lower critical solution temperature, phase separation occurs (disentangled solution) and resolution is lost. The matrix is self-coating and the pore size (which depends on micelle size) is controlled by varying the monomer concentration and temperature. This gel is suitable for the separation of a large range of DNA sizes. The separation of DNA sequencing fragments of BigDye G-labeled M13 of up to 600 bp has been performed (Wei and Yeung, 2001).

CONCLUSION

Capillary gel electrophoresis is a powerful method for the separation and analysis of biopolymers—nucleic acids, their fragments and proteins/peptides. Some gels are routinely used for the determination of molecular mass of the above-mentioned biopolymers, but there is still the need to develop new ones. The major progress made in these new gels has been achieved through the development of copolymers, mainly thermo-responsive polymers that have a temperature-controlled viscosity and form micelle structures.

Acknowledgments

This work was supported by the Czech–Hungarian co-operation programme, the Grant Agency of the Czech Republic, grants nos 203/03/0716, 203/05/2539, the Center for Heart Research 1M6798582302, and by the Research Project AV0Z50110509.

REFERENCES

Albarghouthi MN, Buchholz BA, Doherty EAS, Bogdan FM, Zhou H and Barron AE. Impact of polymer hydrophobicity on the properties and performance of DNA sequencing matrices for capillary electrophoresis. *Electrophoresis* 2001; **22**: 737–747.

Albarghouthi MN, Buchholz BA, Huiberts PJ, Stein TM and Barron AE. Poly-*N*-hydroxyethylacrylamide (polyDuramide™): a novel, hydrophilic, self-coating polymer matrix for DNA sequencing by capillary electrophoresis. *Electrophoresis* 2002; **23**: 1429–1440.

Anastos N, Barnett NW and Lewis SW. Capillary electrophoresis for forensic drug analysis: a review. *Talanta* 2005; **67**: 269–279.

Banks JF. Protein Analysis. In *Advanced Chromatographic and Electromigration Methods in BioSciences*, Tesarova E (ed.). Elsevier: Amsterdam, 1998; 525–573.

Barbier V, Buchholz BA, Barron AE and Viovy J-L. Comb-like copolymers as self-coating, low-viscosity and high-resolution matrices for DNA sequencing. *Electrophoresis* 2002; **23**: 1441–1449.

Barbier V and Viovy J-L. Advanced polymers for DNA separation. *Current Opinion in Biotechnology* 2003; **14**: 51–57.

Buchholz BA, Doherty EAS, Albarghouthi MN, Bogdan FM, Zahn JM and Barron AE. Microchannel DNA sequencing matrices with a thermally controlled 'viscosity switch'. *Analytical Chemistry* 2001; **73**: 157–164.

Buchholz BA, Shi W and Barron AE. Microchannel DNA sequencing matrices with switchable viscosities. *Electrophoresis* 2002; **23**: 1398–1409.

Chankvetadze B, Saito M, Yashima E and Okamoto Y. Enantioseparation using selected polysaccharides as chiral buffer additives in capillary electrophoresis. *Journal of Chromatography A* 1997; **773**: 331–338.

Chiari M, Cretich M, Riva S and Casali M. Performances of new sugar-bearing poly(acrylamide) copolymers as DNA sieving matrices and capillary coatings for electrophoresis. *Electrophoresis* 2001; **22**: 699–706.

Chu B and Liang D. Copolymer solutions as separation media for DNA capillary electrophoresis. *Journal of Chromatography A* 2002; **966**: 1–13.

Cifuentes A, de Frutos M, Santos JM and Diez-Masa JC. Separation of basic proteins by capillary electrophoresis using cross-linked polyacrylamide-coated capillaries and cationic buffer additives. *Journal of Chromatography A* 1993; **655**: 63–72.

Cottet H, Simó C, Vayabourya W and Cifuentes A. Nonaqueous and aqueous capillary electrophoresis of synthetic polymers. *Journal of Chromatography A* 2005; **1068**: 59–73.

Fritz JS, Breadmore MC, Hilder EF and Haddad PR. Use of ionic polymers as stationary and pseudo-stationary phases in the separation of ions by capillary electrophoresis and capillary electrochromatography. *Journal of Chromatography A* 2002; **942**: 11–32.

Garcia-Canas V, Gonzalez R and Cifuentes A. The combined use of molecular techniques and capillary electrophoresis in food analysis. *TrAC Trends in Analytical Chemistry* 2004; **23**: 637–643.

Gilar M, Smisek DL and Cohen AS. Nucleic acids and their constituents. In *Advanced Chromatographic and Electromigration Methods in BioSciences*, Tesarova E (ed.). Elsevier: Amsterdam, 1998; 575–607.

Guttman M, Fules P and Guttman A. Analysis of site-directed mutagenesis constructs by capillary electrophoresis using linear polymer sieving matrices. *Journal of Chromatography A* 2003; **1014**: 21–27.

Hjertén S, Liao J-L and Zhang R. High-performance liquid

chromatography on continuous polymer beds. *Journal of Chromatography* 1989; **473**: 273–275.
Hsieh M-M, Chiu T-C, Tseng W-L and Chang H-T. Analysis of nucleic acids and proteins in capillary electrophoresis and microchip capillary electrophoresis using polymers as additives of the background electrolytes. *Current Analytical Chemistry* 2006; **2**: 17–33.
Issaq HJ, Chan KC, Janini GM, Conrads TP and Veenstra TD. Multidimensional separation of peptides for effective proteomic analysis. *Journal of Chromatography B* 2005; **817**: 35–47.
Kan C-W, Doherty EAS and Barron AE. A novel thermogelling matrix for microchannel DNA sequencing based on poly-*N*-alkoxyalkylacrylamide copolymers. *Electrophoresis* 2003; **24**: 4161–4169.
Kennedy RT, German I, Thompson JE and Witowski SR. Fast analytical-scale separations by capillary electrophoresis and liquid chromatography. *Chemical Review* 1999; **99**: 3081–3113.
Kourkine IV, Ristic-Petrovic M, Davis E, Ruffolo CG, Kapsalis A and Barron AE. Detection of *Escherichia coli* O157:H7 bacteria by a combination of immunofluorescent staining and capillary electrophoresis. *Electrophoresis* 2003; **24**: 655–661.
Li C, Liu X and Meng L-Z. Novel amphiphilic copolymer with pendant tris(trimethylsiloxy)silyl group: synthesis, characterization and employment in CE DNA separation. *Polymer* 2004; **45**: 337–344.
Liang D and Chu B. High speed separation of DNA fragments by capillary electrophoresis in poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) triblock polymer. *Electrophoresis* 1998; **19**: 2447–2453.
Liang D, Song L, Zhou S, Zaitsev VS and Chu B. Poly(*N*-isopropylacrylamide)-g-poly(ethyleneoxide) for high resolution and high speed separation of DNA by capillary electrophoresis. *Electrophoresis* 1999a; **20**: 2856–2863.
Liang D, Zhou S, Song L, Zaitsev VS and Chu B. Copolymers of poly(*N*-isopropylacrylamide) densely grafted with poly(ethylene oxide) as high-performance separation matrix of DNA. *Macromolecules* 1999b; **32**: 6326–6332.
Liang D, Liu T, Song L and Chu B. Mixed triblock copolymers used as DNA separation medium in capillary electrophoresis. *Journal of Chromatography A* 2001; **909**: 271–278.
Lin Y-W, Huang M-J and Chang H-T. Analysis of double-stranded DNA by microchip capillary electrophoresis using polymer solutions containing gold nanoparticles. *Journal of Chromatography A* 2003; **1014**: 47–55.
Linse P. Phase behaviour of poly(ethylene oxide)–poly(propylene oxide) block copolymers in aqueous solution. *Journal of Physical Chemistry* 1993; **97**: 13896.
Liu T, Liang D, Song L, Nace VM and Chu B. Spatial open-network formed by mixed triblock copolymers as a new medium for double-stranded DNA separation by capillary electrophoresis. *Electrophoresis* 2001; **22**: 449–458.
Liu Y, Locke BR, Van Winkle DH and Rill RL. Optimizing capillary gel electrophoretic separations of oligonucleotides in liquid crystalline Pluronic F127. *Journal of Chromatography A* 1998; **817**: 367–375.
Magnusdottir S, Viovy J-L and François J. High resolution capillary electrophoretic separation of oligonucleotides in low-viscosity, hydrophobically endcapped polyethylene oxide with cubic order. *Electrophoresis* 1998; **19**: 1699–1703.
Malmsten M and Lindman B. Self-assembly in aqueous block copolymer solutions. *Macromolecules* 1992; **25**: 5440–5445.
Menchen S, Johnson B, Winnik MA and Xu B. Flowable networks as DNA sequencing media in capillary columns. *Electrophoresis* 1996; **17**: 1451–1459.
Miksik I and Deyl Z. Application of Pluronic copolymer liquid crystals for the capillary electrophoretic separation of collagen type I cyanogen bromide fragments. *Journal of Chromatography B: Biomedical Sciences and Applications* 2000; **739**: 109–116.
Miksik I, Deyl Z and Kasicka V. Capillary electrophoretic separation of proteins and peptides using Pluronic liquid crystals and surface-modified capillaries. *Journal of Chromatography B: Biomedical Sciences and Applications* 2000; **741**: 37–42.
Miksik I, Eckhardt A, Forgacs E, Cserhati T and Deyl Z. The effect of SDS and Pluronic F127 on the electrophoretic separation of protein and polypeptide test mixtures at acid pH. *Electrophoresis* 2002; **23**: 1882–1886.
Miksik I, Charvatova J, Eckhardt A and Deyl Z. Peptide mapping by capillary electrophoresis with Pluronic F127. *Journal of Chromatography B* 2004; **800**: 155–160.
Mortensen K and Talmon Y. Cryo-TEM and SANS Microstructural study of Pluronic polymer solutions. *Macromolecules* 1995; **28**: 8829–8834.
Mortensen K, Brown W and Norden B. Inverse melting transition and evidence of three-dimensional cubatic structure in a block-copolymer micellar system. *Physical Review Letters* 1992; **68**: 2340–2343.
Nakatani M, Shibukawa A and Nakagawa T. High-performance capillary electrophoresis of SDS-proteins using pullulan solution as separation matrix. *Journal of Chromatography A* 1994; **672**: 213–218.
Nakatani M, Shibukawa A and Nakagawa T. Separation mechanism of pullulan solution-filled capillary electrophoresis of sodium dodecyl sulfateproteins. *Electrophoresis* 1996; **17**: 1584–1586.
Petersen JR, Okorodudu AO, Mohammad A and Payne DA. Capillary electrophoresis and its application in the clinical laboratory. *Clinica Chimica Acta* 2003; **330**: 1–30.
Rill RL and Al-Sayah MA. Peptide separations by slab gel electrophoresis in Pluronic F127 polymer liquid crystals. *Electrophoresis* 2004; **25**: 1249–1254.
Rill RL, Liu Y, Van Winkle DH and Locke BR. Pluronic copolymer liquid crystals: unique, replaceable media for capillary gel electrophoresis. *Journal of Chromatography A* 1998a; **817**: 287–295.
Rill RL, Locke BR, Liu Y and Van Winkle DH. Electrophoresis in lyotropic polymer liquid crystals. *PNAS* 1998b; **95**: 1534–1539.
Rodriguez MA and Armstrong DW. Separation and analysis of colloidal/nano-particles including microorganisms by capillary electrophoresis: a fundamental review. *Journal of Chromatography B* 2004; **800**: 7–25.
Sakai-Kato K, Kato M, Nakajima T, Toyo'oka T, Imai K and Utsunomiya-Tate N. Cationic starch derivatives as dynamic coating additives for protein analysis in capillary electrophoresis. *Journal of Chromatography A* 2006; **1111**: 127–132.
Sartori A, Barbier V and Viovy J-L. Sieving mechanisms in polymeric matrices. *Electrophoresis* 2003; **24**: 421–440.
Shambaugh CL, Bodmer J-L, Hsu D and Ranucci CS. Separation and quantification of viral double-stranded RNA fragments by capillary electrophoresis in hydroxyethylcellulose polymer solutions. *Journal of Chromatography A* 2004; **1051**: 161–170.
Song L, Liu T, Liang D, Fang D and Chu B. Separation of double-stranded DNA fragments by capillary electrophoresis in interpenetrating networks of polyacrylamide and polyvinylpyrrolidone. *Electrophoresis* 2001; **22**: 3688–3698.
Sudor J, Barbier V, Thirot S, Godfrin D, Hourdet D, Millequant M, Blanchard J and Viovy J-L. New block-copolymer thermoassociating matrices for DNA sequencing: effect of molecular structure on rheology and resolution. *Electrophoresis* 2001; **22**: 720–728.
Svec F and Tennikova TB. Historical review. In *Monolithic Materials*, Svec F, Tennikova TB and Deyl Z (eds). Elsevier: Amsterdam, 2003; 1–15.
Szantai E, Ronai Z, Szilagyi A, Sasvari-Szekely M and Guttman A. Haplotyping by capillary electrophoresis. *Journal of Chromatography A* 2005; **1079**: 41–49.
Viklund C, Svec F, Fréchet JMJ and Irgum K. Monolithic, 'Molded', porous materials with high flow characteristics for separations, catalysis, or solid phase chemistry: control of porous properties during polymerization. *Chemical Materials* 1996; **8**: 744–750.
Wang Y, Liang D, Hao J, Fang D and Chu B. Separation of double-stranded DNA fragments by capillary electrophoresis using polyvinylpyrrolidone and poly(*N*,*N*-dimethylacrylamide) transient interpenetrating network. *Electrophoresis* 2002; **23**: 1460–1466.
Wang Y, Liang D, Ying Q and Chu B. Quasi-interpenetrating network formed by polyacrylamide and poly(N,N-dimethylacrylamide) used in high-performance DNA sequencing analysis by capillary electrophoresis. *Electrophoresis* 2005; **26**: 126–136.
Wanka G, Hoffmann H and Ulbricht W. Phase diagrams and aggregation behavior of poly(oxyethylene)–poly(oxypropylene)–poly(oxyethylene) triblock copolymers in aqueous solutions. *Macromolecules* 1994; **27**: 4145–4159.
Wei W and Yeung ES. DNA capillary electrophoresis in entangled

dynamic polymers of surfactant molecules. *Analytical Chemistry* 2001; **73**: 1776–1783.

Wu C, Liu T, Chu B, Schneider DK and Graziano V. Characterization of the PEO-PPO-PEO triblock copolymer and its application as a separation medium in capillary electrophoresis. *Macromolecules* 1997; **30**: 4574–4583.

Wu C, Liu T and Chu B. A new separation medium for DNA capillary electrophoresis: self-assembly behavior of Pluronic polyol E99P69E99 in 1X TBE buffer. *Journal of Non-Crystalline Solids* 1998a; **235–237**: 605–611.

Wu C, Liu T and Chu B. Viscosity-adjustable block copolymer for DNA separation by capillary electrophoresis. *Electrophoresis* 1998b; **19**: 231–241.

Zhou S. Separation and detection methods for covalent drug–protein adducts. *Journal of Chromatography B* 2003; **797**: 63–90.

Zhu J and Feng Y-L. Size exclusive capillary electrophoresis separation of DNA oligonucleotides in small size linear polyacrylamide polymer solution. *Journal of Chromatography A* 2005; **1081**: 19–23.