ELSEVIER

Journal of Chromatography B, 699 (1996) 63–75

JOURNAL OF CHROMATOGRAPHY B

Review

Electrophoresis gel media: the state of the art

Pier Giorgio Righetti[a,*], Cecilia Gelfi[b]

[a]*Department of Agricultural and Industrial Biotechnologies, University of Verona, Strada le Grazie, Ca' Vignal 37134 Verona, Italy*
[b]*ITBA, CNR, Via Ampère 56, Milan, Italy*

Abstract

Some unique events have occurred in the last few years which might revolutionize the field of polyacrylamide gel electrophoresis. While it was widely recognized that such matrices could normally be cast with a small pore size distribution, typically of the order of a few nanometers diameter (for protein sieving), recent developments suggest that "macroporous" gels could also be produced in the domain of polyacrylamides. If constraints to chain motion are imposed during gel polymerization, large-pore structures can be grown. Such constraints can originate either from low temperatures or from the presence of preformed polymers in the gelling solution; in both cases, the growing chains are forced to "laterally aggregate" via inter-chain hydrogen bond formation. Upon consumption of pendant double bonds, such bundles are frozen in the three-dimensional space by permanent cross-links. As an additional development, a novel photopolymerization system is described, comprising a cationic dye (methylene blue) and a redox couple (sodium toluene sulfinate, a reducer, and diphenyliodonium chloride, a mild oxidizer). Methylene blue catalysis is characterized by a unique efficiency, ensuring >96% conversion of monomers, even in hydro-organic solvents and in the presence of surfactants, which normally quench or completely inhibit the persulphate-driven reaction. In addition, methylene blue-sustained photopolymerization can be operated in the entire pH 3–10 interval, where most other systems fail. Perhaps the most striking novelty in the field is the appearance of a novel monomer (N-acryloylaminopropanol, AAP) coupling a high hydrophilicity with a unique resistance to alkaline hydrolysis. Given the fact that a poly(AAP) matrix is 500 times more stable than a poly(acrylamide) gel, while being twice as hydrophilic, it is anticipated that this novel chemistry will have no difficulties in replacing the old electrophoretic anticonvective media. The review ends with a glimpse at novel sieving media in capillary zone electrophoresis: polymer networks. Such media, by providing an almost infinite range of pore sizes, due to the absence of a rigid support, allow sieving mechanisms to be operative over a wide interval of particle sizes, even up to genomic DNA. Viscous solutions of polymer networks, made with the novel poly(AAP) chemistry, allow repeated use of the same separation column, well above 50 injections. Silica-bound poly(AAP) chains provide effective quenching of electrosmosis and >200 analyses by isoelectric focusing. © 1996 Elsevier Science B.V.

Keywords: Reviews; Gel media; N-Acryloylaminoethoxyethanol; N-Acryloylaminopropanol

Contents

*Corresponding author. Address for correspondence: L.I.T.A., University of Milano, via Fratelli Cervi 93, Segrate 20090 (Milano), Italy.

PII S0378-4347(96)00207-1

1. Introduction

We will attempt here to summarize the evolution of electrophoretic matrices, from the early days in the 1950s to modern times. Perhaps one of the earliest reports on the use of hydrophilic support media comes from the work of Gordon et al. [1] who proposed in 1950 the use of 3% agar in protein separation. The technique became widely popularized through the work of Grabar and Williams [2], who added to it a unique depth of field; analyte recognition via immuno-detection. In 1955, Smithies [3] introduced starch gel electrophoresis (typically soluble potato starch at ca. 15% concentration in borate buffer). The technique became very popular among geneticists, who spent most of their time in the late fifties and early sixties preparing their own soluble starch suitable for protein electrophoresis. The reason for this excitement came from the discovery, via starch block electrophoresis, by Smithies and Walker [4] of the first polymorphism in human proteins; that of haptoglobins, which were resolved into three types, i.e., 1–1, 2–1 and 2–2. In the early sixties, polyacrylamide matrices were introduced by Raymond and Weintraub [5] and were greatly popularized by Davis [6], Ornstein [7] and Hjertèn [8], due to the high resolution obtained by the use of discontinuities in buffers, porosity and counterions. Today, however, notwithstanding the hundreds of support media available in chromatography [9], in electrophoresis only three types of matrices are widely used; polyacrylamides, agaroses and cellulose acetate [10,11].

Agarose is typically used for electrophoresis of DNA fragments, due to supercoiled fibers with a radius of 20–30 nm, generating pore sizes of 200 nm and higher [12]. Conversely, polyacrylamide gels, having pore size values ranging from a few nm up to 15–20 nm in highly diluted formulations [13,14], are most efficient in protein separations. Polyacrylamide gels suitable for electrophoretic applications have been prepared with a variety of initiators and conditions (temperature, additives, concentrations of monomers and cross-linking) [15]. However, in the most common cases, a radical polymerization reaction of acrylamide and N,N′-methylenebisacrylamide (Bis), catalyzed by N,N,N′,N′-tetramethylethylenediamine (TEMED) and ammonium peroxydisulphate (APS), leads to formation of a gel in which the conversion from monomer to polymer reaches ca. 90% within the first 15 min [16]. Perhaps the most interesting innovation in polymerization chemistry has been the introduction of methylene blue photopolymerization, coupled to the use of the redox system sodium toluene sulfinate and diphenyl iodonium chloride, allowing for very high conversion efficiencies (>96%) over the pH 3–10 scale [17–19]. Yet, over the years, the choice of anticonvective support has been substantially limited to the systems discovered in the early sixties, and gel optimization work was devoted to a relatively large number of cross-linkers, imparting special properties to the matrix for different fractionation purposes [20].

As will be shown below, major progress has been made recently in gel matrices. Some of these results have been summarized already in recent reviews [10,11,21,22].

2. Macroporous polyacrylamides: polymer-induced lateral aggregation

Recently we chanced upon a curious phenomenon; when gelling a polyacrylamide in the presence of another polymer [e.g., poly(ethylene glycol) (PEG)],

polyvinyl pyrrolidone, hydroxymethyl cellulose) turbid gels were produced. The turbidity was a function of both length and concentration of the polymer in the gelling solution. In solutions of PEG ranging from 2000 Da up to 20 000 Da, longer polymer chains induce this transition at much lower concentrations (ca. 1.2% in 20 kDa PEG, vs. >10% in 2 kDa PEG). We concluded that the preformed polymer present in solution was eliciting bundling of the growing polyacrylamide chains [23]. Yet, the phenomenon was unique in that, contrary to previous studies, this transition was obtained at regular percentages of cross-linker (C) (in fact, barely 4% C). Additional studies suggested that the main phenomenon responsible for this bundling event was the formation of inter-chain hydrogen bonds, that occurred during the growth of the polymer and just prior to the cross-linking reaction. In fact, it was shown that urea and temperature (typical H-bond breakers), either alone or in association, would completely abolish the process, whereas an agent such as tetramethyl urea could not hamper it [23]. The hypothesis of inter-chain H-bond formation was further corroborated by the following observations; (a) extremes of pH would quench the process; (b) the presence of monomeric ethylene glycol and even glycerol (both at concentrations >25%) would also hamper gel turbidity; (c) finally, production of mixed-monomer matrices (e.g., acrylamide admixed with either N-methylacrylamide or N,N-dimethylacrylamide) also interfered with gel turbidity [25]. That such highly turbid matrices could have a high pore size was proven by scanning electron microscopy (which detected large fibre bundles, of ca. 200 to 300 nm delimiting "holes" of equivalent diameter) [24]. An additional, direct, proof came from the experiment of Fig. 1: The gels shown therein demonstrate the possibility of creating a porosity gradient at a fixed concentration of monomers throughout, simply by preparing the gel in the presence of a gradient of "laterally aggregating" agent. It is seen that, in a conventional 6%T, 4%C matrix, large DNA fragments (21 kbp) cannot even penetrate the gel meshwork and are trapped at the deposition site. However, when the same gel is polymerized in a 0–1% 10 kDa PEG gradient, the same 21 kbp fragments penetrate 15% down the gel length and, in a gel cast against a 0–2% 10 kDa PEG

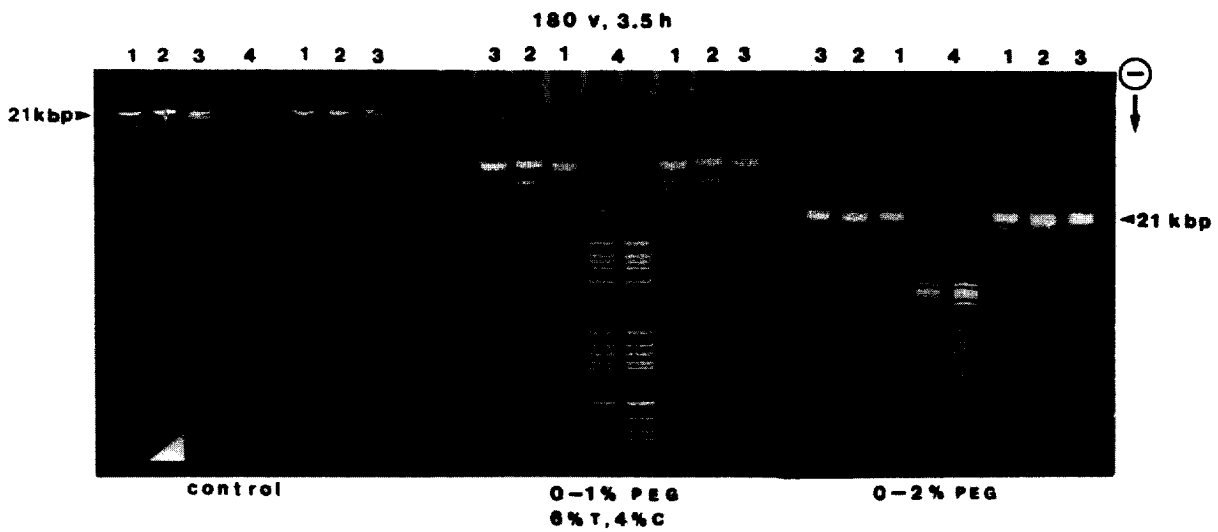


Fig. 1. Fractionation of DNA fragments in control and "laterally aggregated" gels. All gels were 6%T, 4%C in the absence (control, left) or presence of a linear gradient from 0 to 1% (central) or from 0 to 2% (right panel) of 10 kDa PEG. All gels were run for 3.5 h at 180 V in Tris–borate–EDTA buffer, pH 8.3. Staining was performed by dye intercalation with ethidium bromide. Note how the 21 000 bp fragment is trapped at the origin in control gels, whereas it migrates down 15% of the gel length in the presence of the 0–1% PEG gradient (central gel) and 30% of the gel length in the 0–2% PEG gradient (right gel). Samples: 1: M_r marker III; 2: M_r marker II; 3: M_r marker I and 4: M_r marker V (from Righetti et al., see Ref. [13], with permission).

gradient, it migrates down 30% of the gel length. We additionally have proven that, in such matrices, one can focus immunoglobulin M (M_r ca. $1 \cdot 10^6$) [24]. Furthermore, small-angle laser light scattering measurements, performed during the evolution of this gelation process, have proven that the large pores are generated by a microsegregation process caused by competition between gelation and phase separation of the polymer solution. This separation occurs by spinodal decomposition (see Fig. 2), and the size of the pores is determined by the stage at which the decomposition is stopped by the gelation process [25]. Most interestingly, this microsegregation process has been applied to agarose gels as well, with the result of producing matrices with pores as large as 10 μm [26].

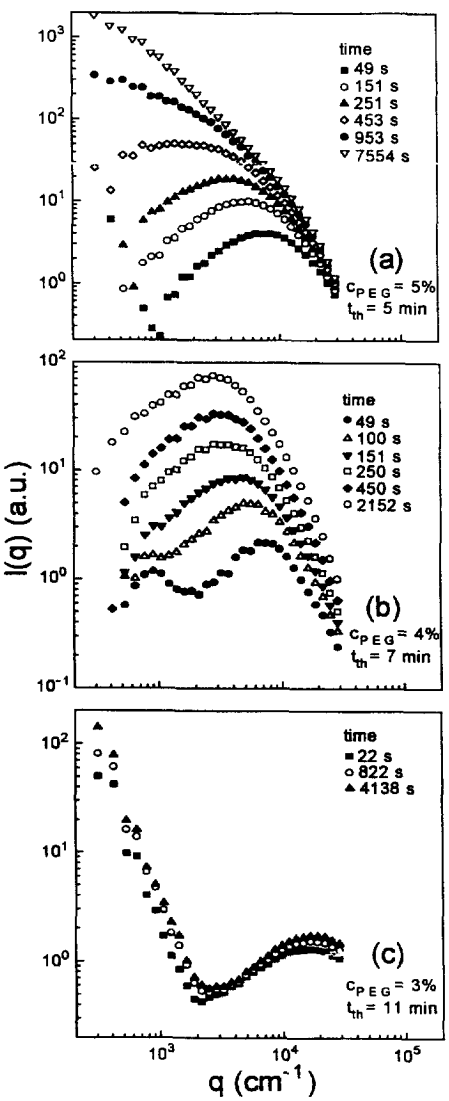


Fig. 2. Time evolutions of the scatttering intensity distribution for gelling solutions at different PEG concentrations: (a) 5%; (b) 4%; (c) 3%. Photopolymerization has been induced according to the threshold exposure criterium. The various time points indicate the beginning of the scattering data acquisition after the photopolymerization exposure t_{th} (from Asnaghi et al., see Ref. [25], with permission).

3. Macroporous polyacrylamides: temperature-induced aggregation

In our search for porous matrices, we noticed another phenomenon that could be correlated to the "lateral aggregation" event described above in the case of preformed polymers. We were trying to standardize a photopolymerization system, comprising 100 μ*M* methylene blue, in the presence of a redox system, 1 m*M* sodium toluene sulfinate (a reducer) and 50 μ*M* diphenyliodonium chloride (an oxidizer) [27]. We noticed that, when photopolymerization was started at 2°C, the matrix formed was completely clear at the gelation point. However, if the polymerization was continued, after the gel point, at 2°C, the gel became progressively turbid, with the Tyndall effect plateauing after 50 min of reaction. On the contrary, if, after gelation at 2°C, the reaction was continued at 50°C, a fully transparent matrix was obtained. By plotting the turbidity vs. the polymerization temperature, a melting point at 28°C was obtained (see Fig. 3), suggesting that, by gelation at 2°C, the nascent chains formed clusters held together by hydrogen bonds, a process mimicking "lateral aggregation" as induced by gelling in the presence of exogenous polymers, such as PEG. Additionally, one must assume that in the growing polymer there would be quite a number of pending, but still unreacted, double bonds. Upon maturation, these pendant bonds would be consumed. Thus, if the reaction was started and continued for the whole time at 2°C, the nascent chains would first form clusters held together by H-bonds and subsequently lock such bundles in the three-dimensional space as the pendant double bonds in the chains were allowed

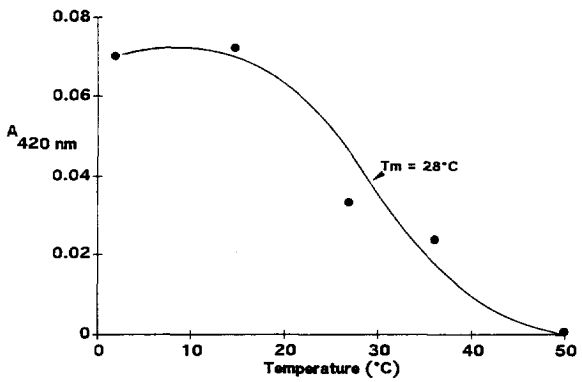


Fig. 3. Determination of the melting point (T_m) of the turbid gel phase upon polymerization at different temperatures (2–50°C). The absorbance plateau values upon gel ripening are plotted against the respective polymerization temperatures. The curve was traced by a sigmoidal curve-fitting program and the T_m was assessed as 28°C (from Righetti and Caglio, see Ref. [27], with permission).

to progressively react. Thus, the following model has emerged; when no constraints are imposed on the growing polymer, the gel will form a domain of a random meshwork of fibres. However, if there are motion constraints, such as those caused by a preformed polymer in solution, chain bundles will form, producing a large-pore gel. In the presence of another motion constraint, low temperatures, bundling also occurs, with the formation of large-pore matrices. Without fully realizing this chain of events, and their inter-connections, we had already reported in 1981 that indeed the gelling temperature could substantially alter the pore size of the growing matrix [28].

4. Novel polymerization chemistry

We have recently described a photopolymerization system, comprising a cationic dye (methylene blue, MB) and a redox couple (sodium toluene sulfinate, a reducer, and diphenyliodonium chloride, a mild oxidizer) [17]. MB-driven catalysis offered highly reproducible gel points (180±8 s at 30°C) and produced gels with better visco-elastic properties than equivalent gels chemically initiated with the standard redox couple, persulphate and TEMED [17]. In a subsequent theoretical study, it was found that excellent conversion efficiency (in all cases >96% incorporation of monomers into the growing polymer) could be obtained, provided that the correct levels of dye and proper light intensity were used [18]. Paradoxically, if the levels of sensitizer or incident light intensities used were too high, the rate of dye consumption was found to be too high compared to monomer incorporation, so that dye depletion might occur prior to chain elongation, and the reaction would then suddenly cease. Yet, when correctly used, photopolymerization was found to give the best conversion efficiency among different initiation processes [18]. In a third article of the series, another feature of MB-driven catalysis was discovered; a very high conversion efficiency was guaranteed by this system over the entire pH 4–10 range. Conversely, riboflavin-driven polymerization offered a maximum only over a narrow pH window (pH 6.2 to 6.5), the reaction slowly declining at acidic pH values and being strongly quenched at progressively alkaline values, with complete inhibition at pH 10. A specular behaviour could be demonstrated in persulphate–TEMED initiation; complete inhibition at pH 4, with highest conversions in the pH 7–10 interval (see Fig. 4). Two other

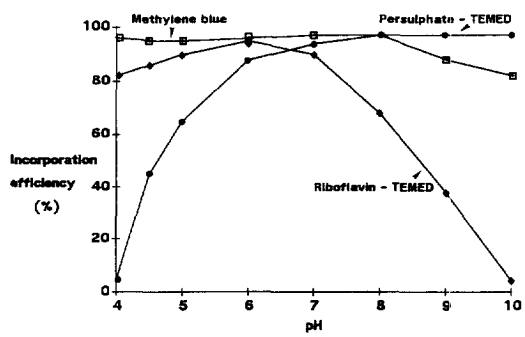


Fig. 4. Incorporation efficiencies of the systems persulphate–TEMED, riboflavin–TEMED and methylene blue (MB) vs. pH of the gelling solution. The conversion was assessed by eluting the ungrafted monomers from the gel phase, followed by separation and quantitation of free monomers by capillary zone electrophoresis. The molar ratio of the monomers in the gel vs. the free liquid phase is taken to represent the incorporation efficiency. Note that in the riboflavin system, optimum incorporation occurs only in the narrow pH 6.2–6.5 window; in persulphate, the pH optimum is in the pH 7.5–10 interval, whereas in MB-catalysis excellent conversion can be obtained in the entire pH 4–10 range (modified from Caglio and Righetti, see Ref. [29], with permission).

systems investigated (ascorbic acid, ferrous sulphate and hydrogen peroxide in one case, and persulphate, TEMED and hydrosulphite, in another case) exhibited only modest conversion efficiencies [19]. In additional articles, we studied the incorporation kinetics of both co-monomers in the proximity of the critical point [27], the effect of a series of organic solvents on the reaction efficiency [29] and finally the effect of different surfactants in quenching polymerization [30]. In all cases, MB-driven polymerization had a superior performance to any other initiator system; yet, not many instances of its use can be found in the literature. One reason could be the fact that, apparently, MB is covalently incorporated at one extremity of the growing polyacrylamide chains and this could induce hydrophobic interaction of the dye with proteins. It has been suggested [31] that the amount of dye should be lowered by a factor of two or three, since even at this dilution the polymerization reaction is just as efficient.

5. Novel monomer chemistry

As stated in the Section 1, the pair of monomers that have attained the greatest popularity have been acrylamide coupled to the cross-linker N,N′-methylenebisacrylamide. However, several defects of such a matrix have been noticed upon prolonged use. Among them are (a) instability at alkaline pH values, resulting in hydrolysis of the amido bond with the formation of polyacrylate; (b) the limited range of pore sizes (from 2–3 up to ca. 20–30 nm) in highly diluted matrices and (c) the low efficiency of the standard redox pair of initiators, peroxodisulfate and TEMED. Additionally, this redox couple is able to oxidize amino buffers to N-oxides; the latter, in turn, can oxidize -SH residues in proteins [32]. A remedy to this last problem is now available with MB-photocatalysis which is, by its nature, non-oxidizing.

Only in the eighties were N-substituted polyacrylamide matrices proposed. Boschetti [33] first introduced Trisacryl (N-acryloyl-2-amino-2-hydroxymethyl-1,3 propane diol; NAT) matrices, but only as beads in chromatography for the production of a number of ion-exchangers (CM, DEAE and SP-Trisacryl). Trisacryl, the most hydrophilic monomer ever reported [34], due to its large mass, also had the advantage of producing highly porous gels. In spite of its advantages, its use in isoelectric focusing [35,36] and DNA separations [37] cannot be advocated, due to its inherent instability in alkaline solutions (it degrades with zero-order kinetics!) [34]. Subsequently, acrylamido sugars, such as N-acryloyl (or methacryloyl)-1-amino-1-deoxy-D-glucitol, or the corresponding D-xylitol derivative, were proposed by Kozulic [38]. Acrylic monomers bearing mono-saccharide residues are known to produce highly cohesive and hydrophilic polymers. This type of polymer has a rather hydrophobic backbone (a pure alkane moiety) but the pendant saccharides render their surface very hydrophilic and thus compatible with protein separations. Additionally, the molecular mass of the monomer, which is several times higher than that of acrylamide, allows production of matrices of increased porosity. However, acrylamido-sugars are also extremely unstable for the same electronic and steric reasons that apply to Trisacryl. In 1984, gels derived from polymerization of acryloyl morpholine cross-linked with bisacrylyl piperazine (BAP) were introduced [39,40]. These gels exhibited the interesting feature of being amphiphilic and thus compatible with mixed polar solvents. The resistance of these monomers to alkaline hydrolysis, while somewhat higher than that of acrylamide, is however limited by the fact that the nitrogen involved in the amido bond is inserted into a morpholino ring and later studies demonstrated that rigid ring structures were not so efficient in protecting the adjacent amido bond [41]. Although the cross-linker, BAP, has found applications in the production of gels to be silver stained, as it gives a clear background, gels based on acryloyl morpholine were not adopted, probably due to their rather hydrophobic characters. In fact, in the original report, only an acryloyl morpholine–acrylamide copolymer (in a 1:1 ratio) was found to give acceptable protein patterns.

In 1989, a broad class of N-mono and di-substituted acrylamido derivatives was introduced as electrophoretic support media under the trade-name of Hydrolink [42–44]. However, the monomer preferentially used in Hydrolink formulations turned out to be N,N-dimethylacrylamide (DMA), generally copolymerized with hydroxyethyl methacrylate. The peculiar property of this matrix is its amphiphilic

character, which imparts to the polymer the ability to reswell in a number of polar organic solvents. This feature, although promising for electrophoresis in organic solvents, indicates that DMA possesses a marked hydrophobic character, which limits its applicability only to DNA separations. In fact, when used in protein separations, it gave irreversible adsorption at the application site, sample precipitation and pronounced smears (Righetti et al., unpublished). Nevertheless, modified polyacrylamide matrices were independently reported by Perrella et al. [45] for focusing hemoglobins at sub-zero (−20°C) temperatures. These authors utilized copolymers of acrylamide–methacrylate or acrylamide–ethylacrylate, which are able to stand temperatures as low as −30 to −40°C without a glass transition in the matrix. In 1992, Zewert and Harrington [46,47], proposed a poly(ethylene glycol) methacrylate–acrylamide copolymer for electrophoresis, as an alternative to agarose and polyacrylamides. Although these authors additionally reported its use for separating hydrophobic proteins [48], their results only gave poor smears of zeins (maize storage proteins, soluble only in 50% alcohol), suggesting that the hydrophobicity of this matrix is still too high to be compatible with protein electrophoresis. Additionally, as this matrix is a methacrylate ester, rapid hydrolysis is to be expected upon standard electrophoresis (which routinely employs buffers with a pH of 8.5 to 9.5). At just about the same time, Patton et al. [49] proposed Duracryl, a mechanically strong and elastic polyacrylamide-based medium, for use in isoelectric focusing (IEF) and sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE). Upon addition of appropriate initiators, Duracryl forms a "polymer reinforced" polyacrylamide possessing improved mechanical strength (the composition, however, has not been divulged). As a latest addition, Chiari et al. [50] recently reported agarose–polyacrylamide mixed-bed formulations. This last matrix, although of the same type as the ethylenically unsaturated polysaccharide resins already described by Nochumson [51], differs from the latter in that it contains a highly controlled amount of allylglycidyl double bonds, typically one residue every disaccharide unit, which leads to a highly reproducible matrix and controlled pore size and degree of cross-linking.

This was the electrophoretic panorama when, in 1994, Righetti's group introduced a novel monomer, N-acryloylaminoethoxyethanol (AAEE) combining the two most wanted properties; very high hydrophilicity coupled to an extraordinary stability to alkaline hydrolysis [52]. This monomer has been used in DNA electrophoresis in capillaries [53], as a coating of the silica wall [54], in isoelectric focusing analysis under very alkaline conditions [55,56] and as an inert base for isoelectric membranes [57].

With the discovery of the AAEE monomer, it would appear that the search for novel matrices has finally come to an end. Much to our dismay, we recently found some disturbing negative features we had no way of predicting: While the polymer offers the unique performance outlined above, the AAEE monomer suffers from a peculiar instability; upon storage (even in the presence of radical inhibitors), it could suddenly auto-polymerize. If kept concentrated (routinely we store it as a 50:50 (v/v) water solution, which minimizes the problem) auto-polymerization is almost guaranteed. Simò-Alfonso et al. [58,59] and Gelfi et al. [60] have explored this phenomenon and found a degradation pathway of this monomer, called "1–6 H-transfer", by which the C_1 (on the double bond site), by constantly ramming against the C_6, next to the ether oxygen (O_7, which in fact favours the transfer of the hydrogen atom by C_1), produces radicals that add to the monomer more efficiently, favouring auto-polymerization and cross-linking. A number of novel monomers were thus proposed [58], aimed at eliminating this random auto-polymerization process while maintaining the other unique characteristics of AAEE. One of them, N-acryloylaminopropanol (AAP) [59], was found to offer all the special qualities of AAEE, without the noxious aspects of auto-polymerization. Additionally, a synthetic route was optimized [58], yielding an essentially pure product in the single reaction step, with a yield of >99% and an equivalent purity (>99%). The synthesis consists of reacting acryloyl chloride at −40°C in presence of a two-fold molar excess of amino propanol and with ethanol (instead of methanol) as the solvent. We can thus summarize the panorama given above. In Fig. 5, we list a number of cross-linkers proposed so far (their properties are listed in [20]); in Fig. 6, a list of different monomers developed after acrylamide is given; in

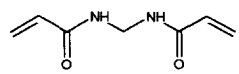
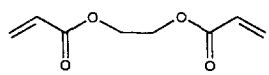
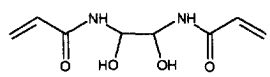
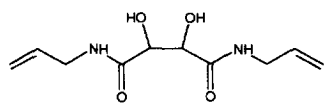
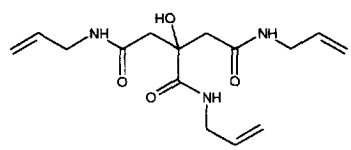
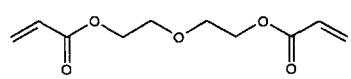
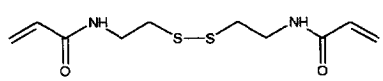
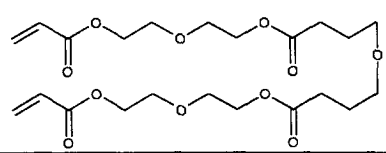
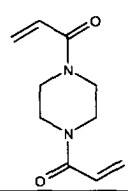
Name	Chemical formula	Mr	Chain Length
N,N'-Methylene bisacrylamide (Bis)		154	9
Ethylene diacrylate (EDA)		170	10
N,N'-(1,2-Dihydroxyethylene) bisacrylamide (DHEBA)		200	10
N,N'-Diallyltartardiamide (DATD)		228	12
N,N',N''-Triallyl citric triamide (TACT)		309	12-13
Polyethylene glycol diacrylate 200 ($PEGDA_{200}$)		214	13
N,N'-Bisacrylyl cystamine (BAC)		260	14
Polyethylene glycol diacrylate 400 ($PEGDA_{400}$)		400	25
N-Bis-acrylyl-piperazine (BAP)		194	10

Fig. 5. Chemical structure of some cross-linkers (modified from Righetti et al., see Ref. [20], with permission).

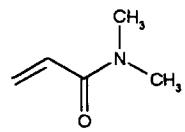
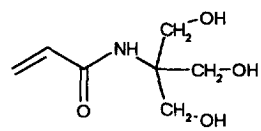
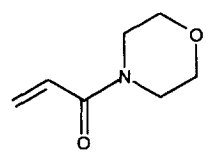
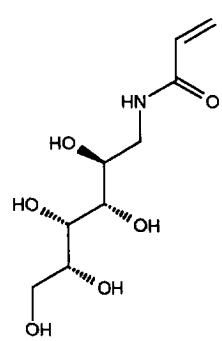
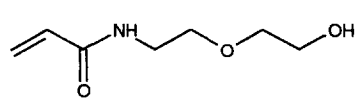
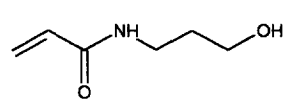
Name	Chemical formula	Mr
N,N-Dimethyl acrylamide (DMA)		99
N-Acryloyl-2-amino-2-hydroxy methyl-1,3-propane (Trisacryl)		175
N-Acryloyl morpholine		141
N-Acryloyl-1-amino-1-deoxy D glucitol		235
N-Acryloyl amino ethoxy ethanol		159
N-Acryloyl amino propanol		129

Fig. 6. Chemical structure of some N-substituted acrylamido monomers.

Fig. 7a, a schematic representation of allyl glycidyl derivatized agarose is offered; in Fig. 7b, an analogous reaction of allyl glycidyl ether with a branched polymer, dextran, is shown.

6. Polymer networks

A long time ago, De Gennes [61] and Bode [62,63] envisaged the possibility of sieving macro-

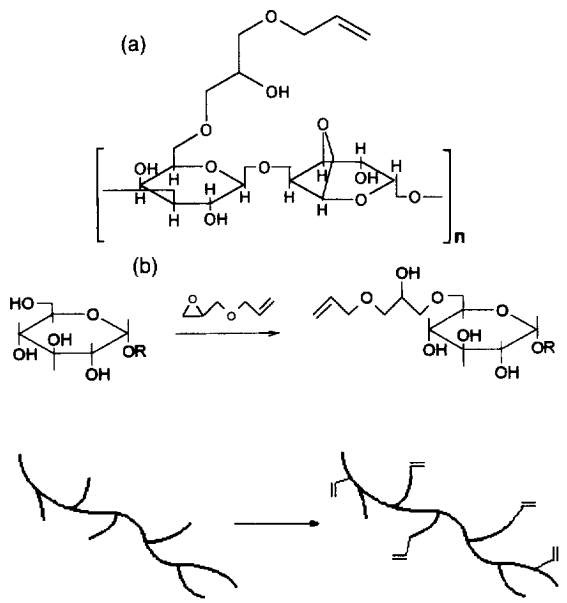


Fig. 7. (a) Schematic representation of allyl glycidyl derivatized agarose (from Chiari et al., see Ref. [50], with permission). (b) Chemical structure of the reaction product between allyl glycidyl ether and the branched polymer, dextran (from Kozulic, see Ref. [38], with permission).

molecules (both nucleic acids and proteins) in polymer networks, i.e. in entangled solutions of linear or branched polymers, in the absence of a rigid network structure, as exists in a truly cross-linked matrix, as polyacrylamide. While the technique did not gain momentum at its inception, it is now becoming quite popular with the advent of capillary zone electrophoresis (CZE). This is due to some simple reasons: First of all, the separation chamber (a capillary) is particularly well suited for holding these viscous polymer solutions in situ; secondly, while it is extremely difficult to fill a capillary with a cross-linked gel, while avoiding air bubbles and all sorts of pestiferous phenomena, this procedure is facilitated in polymer networks; thirdly, viscous polymer solutions (below a viscosity of 100 mPa s) [64] can be replenished easily in the capillary, thus providing a fresh environment for each separation. It is of interest to compare an entangled polymer solution mesh in contrast with a cross-linked gel mesh. The former is a spatial property, being temporarily transient and not a permanent state [65]. A given entangled mesh persists at best up to the relaxation time of the polymer chains constituting the mesh. The residence time of analyte molecules in this mesh is controlled by the size and electrophoretic mobility of the analyte, the mesh size of the network, and the imposed electric field strength. According to either the Ogston model or the reptation mechanism, in order to achieve good resolution, the relaxation time of the entangled polymer solution should be orders of magnitude greater than the residence time of the analyte molecule. In this manner, the mesh is maintained during transit of the analyte, so that the matrix serves as an effective sieving medium. One interesting property of entangled polymer meshworks is the fact that, no matter how large the size of the analyte, even when trapped in a polymer string, it can force it open and pass through. Thus, one of the fundamental characteristics of polymer networks is that there is an almost infinite gradation of pore sizes: Since the pores are dynamic, and not fixed in a rigid reticulum, any object, no matter how large, can force its way through the meshwork (thus, a new migration mechanism has now been described, based on the concept of "constraint release") [66]. In electrophoresis in entangled polymer solutions, it has been predicted that, for a given high molecular mass polymer forming the network, the size of the largest DNA than can be separated increases roughly linearly with the viscosity of the solution [66]. One important practical implication of electrophoresis in polymer networks is that there is essentially no trapping or precipitation of sample at the injection port (even in PCR-amplified fragments in the presence of large genomic material), a drawback that always occurs in gel slabs, in the presence of a cross-linked matrix. In addition to the problems of instability of conventional polyacrylamide, due to hydrolysis of pendant amido groups, this sample precipitation in the application wells is another important cause that renders the re-use of a gel matrix impractical, which for all practical purposes can be regarded as a disposable, single-shot column. Conversely, polymer networks [especially when utilizing poly(AAP) or poly(AAEE) matrices] allow the use of the same column for well over 50 sample injections [67]. A nice example of the separation power of viscous polymer solutions is given in Fig. 8. It displays an SDS electropherogram of six protein standards, run in 4% poly(vinyl alcohol) (PVA) as

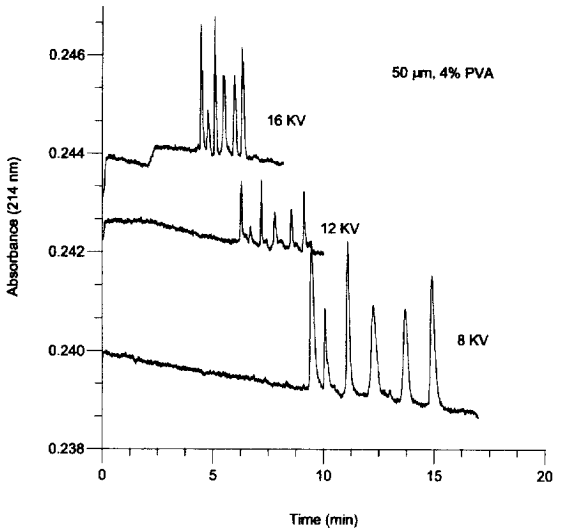


Fig. 8. SDS electropherograms (214 nm readings) of six protein standards. Peaks (from left to right): α-lactalbumin, M_r 14 400; trypsin inhibitor, M_r 20 100; carbonic anhydrase, M_r 30 000; ovalbumin, M_r 43 000; bovine serum albumin, M_r 67 000; phosphorylase b, M_r 94 000. Sieving matrix, 4% PVA; capillary, 34 cm×50 m I.D. Upper trace, 16 kV run; middle trace, 12 kV run; lower trace, 8 kV run (from Simò-Alfonso et al., see Ref. [68], with permission).

the sieving polymer. It is seen that, at high voltages (16 kV), the entire train of zones is eluted in 5 to 7 min. A few features are worth discussing: (a) the great stability of the base-line; (b) the high sensitivity of detection, due to the possibility of reading the absorbance at 214 nm, where proteins absorb ten times more than at 280 nm; and (c) the base-line resolution of all peaks. For DNA separations, we have also produced short-chain polyacrylamides, of low viscosity and optimized chain length, which can be easily replenished in the capillary when exhausted [68,69].

7. Conclusions

There has been marked progress in the last few years in polyacrylamide chemistry, which we can thus summarize:

1. The possibility of gelling "macroporous" matrices by exploiting different ways of forming lateral aggregations, polymer- and temperature-induced;
2. the possibility of high-conversion gelling conditions, by adopting photopolymerization with methylene blue;
3. the discovery of novel monomers, such as N-acryloylaminoethoxyethanol and N-acryloylaminopropanol, combining high hydrophilicity with extreme hydrolytic stability;
4. the possibility of sieving in polymer networks, a most versatile "dynamic matrix" in capillary zone electrophoresis of macromolecules.

It is hoped that the versatility of present-day matrices will meet the needs of most users in todays ever-growing field of electrophoretic applications.

8. Abbreviations

AAEE	N-Acryloylaminoethoxyethanol
AAP	N-Acryloylaminopropanol
BAP	Bisacrylylpiperazine
CM	Carboxymethyl
CZE	Capillary zone electrophoresis
DEAE	Diethylaminoethyl
DMA	Dimethylacrylamide
MB	Methylene blue
PEG	Poly(ethylene glycol)
PVA	Poly(vinyl alcohol)
SDS–PAGE	Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
SP	Sulfopropyl
TEMED	N,N,N′,N′-Tetramethylethylenediamine

Acknowledgements

Supported in part by grants from Consiglio Nazionale delle Ricerche (CNR, Roma) Comitati Chimica (Progetto Strategico Tecnologie Chimiche Innovative, ST74) and Medicina e Biologia (Comitato Tecnologico), by Radius in Biotechnology (ESA, Paris), by Agenzia Spaziale Italiana (ASI, Roma) and by the European Community, BioMed-2 (Human Genome Sequencing).

References

[1] A.H. Gordon, B. Keil, K. Sebasta, O. Knessl and F. Sorm, Coll. Czech. Chem. Commun., 15 (1950) 1–8.
[2] P. Grabar and C.A. Williams, Jr., Biochim. Biophys. Acta, 17 (1955) 67–77.
[3] O. Smithies, Biochem. J., 61 (1955) 629–635.
[4] O. Smithies and N.F. Walker, Nature, 176 (1955) 1265–1266.
[5] S. Raymond and L. Weintraub, Science, 130 (1959) 711–712.
[6] B.J. Davis, Ann. N.Y. Acad. Sci., 121 (1964) 404–427.
[7] L. Ornstein, Ann. N.Y. Acad. Sci., 121 (1964) 321–349.
[8] S. Hjertèn, J. Chromatogr., 11 (1963) 66–70.
[9] R.E. Majors, LC·GC Int., 4 (19) 10–18; ibid. 5 (1992) 11–21; ibid. 6 (1993) 276–288 and 745–753; ibid. 7 (1994) 190–203; ibid. 8 (1995) 266–283.
[10] P.G. Righetti, J. Biochem. Biophys. Methods, 19 (1989) 1–20.
[11] P.G. Righetti, J. Chromatogr. A, 698 (1995) 3–17.
[12] I.T. Norton, D.M. Goodall, K.R.J. Ansten, E.R. Morris and D.A. Rees, Biopolymers, 25 (1986) 1009–1029.
[13] P.G. Righetti, S. Caglio, M. Saracchi and S. Quaroni, Electrophoresis, 13 (1992) 587–595.
[14] E. Wenisch, P. de Besi and P.G. Righetti, Electrophoresis, 14 (1993) 583–590.
[15] A.T. Andrew, Electrophoresis, Theory, Techniques and Biochemical and Clinical Applications, Clarendon Press, Oxford, 1986.
[16] A. Chrambach and D. Rodbard, Science, 172 (1971) 440–451.
[17] T. Lyubimova, S. Caglio, C. Gelfi, P.G. Righetti and T. Rabilloud, Electrophoresis, 14 (1993) 40–50.
[18] T. Lyubimova and P.G. Righetti, Electrophoresis, 14 (1993) 191–201.
[19] S. Caglio and P.G. Righetti, Electrophoresis, 14 (1993) 554–558.
[20] P.G. Righetti, B.C.W. Brost and R.S. Snyder, J. Biochem. Biophys. Methods, 4 (1981) 347–363.
[21] P.G. Righetti, Bio. It., 2 (1995) 20–32.
[22] M. Chiari and P.G. Righetti, Electrophoresis, 16 (1995) 1815–1829.
[23] P.G. Righetti, S. Caglio, M. Saracchi and S. Quaroni, Electrophoresis, 13 (1992) 587–595.
[24] E. Wenisch, P. de Besi and P.G. Righetti, Electrophoresis, 14 (1993) 583–590.
[25] D. Asnaghi, M. Giglio, A. Bossi and P.G. Righetti, J. Chem. Phys., 102 (1995) 9763–9769.
[26] R. Charlionet, L. Levasseur and J.J. Malandain, Electrophoresis, 17 (1996) 58–66.
[27] P.G. Righetti and S. Caglio, Electrophoresis, 14 (1993) 573–582.
[28] C. Gelfi and P.G. Righetti, Electrophoresis, 2 (1981) 220–228.
[29] S. Caglio and P.G. Righetti, Electrophoresis, 14 (1993) 997–1003.
[30] S. Caglio, M. Chiari and P.G. Righetti, Electrophoresis, 15 (1994) 209–214.
[31] T. Rabilloud, M. Vincon and J. Garin, Electrophoresis, 16 (1995) 1414–1422.
[32] M. Chiari, C. Micheletti, P.G. Righetti and G. Poli, J. Chromatogr., 598 (1992) 287–297.
[33] E. Boschetti, in P.D.G. Dean, W.S. Johnson and F.A. Middle (Editors), Affinity Chromatography, IRL Press, Oxford, 1985, pp. 11–15.
[34] C. Gelfi, P. De Besi, A. Alloni and P.G. Righetti, J. Chromatogr., 608 (1992) 333–341.
[35] P.G. Righetti, C. Gelfi, M.L. Bossi and E. Boschetti, Electrophoresis, 8 (1987) 62–70.
[36] M. Kozulic, B. Kozulic and K. Mosbach, Anal. Biochem., 163 (1987) 506–512.
[37] M. Kozulic, K. Mosbach and M. Pietzak, Anal. Biochem., 170 (1988) 478–484.
[38] B. Kozulic, U.S. Patent 5,371,208 (1994).
[39] G. Artoni, E. Gianazza, M. Zanoni, C. Gelfi, M.C. Tanzi, C. Barozzi, P. Ferruti and P.G. Righetti, Anal. Biochem., 137 (1984) 420–428.
[40] G. Vecchio, P.G. Righetti, M. Zanoni, G. Artoni and E. Gianazza, Anal. Biochem., 137 (1984) 410–419.
[41] M. Chiari, C. Ettori and P.G. Righetti, J. Chromatogr., 449 (1991) 119–131.
[42] P.G. Righetti, M. Chiari, E. Casale, C. Chiesa, T. Jain and R.G.L. Shorr, J. Biochem. Biophys. Methods, 19 (1989) 37–50.
[43] C.L. Smith, C.M. Ewing, M.T. Mellon, S.E. Kane, T. Jain and R.G.L. Shorr, J. Biochem. Biophys. Methods, 19 (1989) 51–64.
[44] C.L. Smith, C.M. Ewing, M.T. Mellon, R.G.L. Shorr and T. Jain, J. Biochem. Biophys. Methods, 19 (1989) 65–74.
[45] M. Perrella, L. Cremonesi, I. Vannini Parenti, L. Benazzi and L. Rossi Bernardi, Anal. Biochem., 104 (1980) 126–132.
[46] T.E. Zewert and M.G. Harrington, Electrophoresis, 13 (1992) 817–824.
[47] T.E. Zewert and M.G. Harrington, Electrophoresis, 13 (1992) 824–831.
[48] T.E. Zewert and M.G. Harrington, Electrophoresis, 15 (1994) 195–199.
[49] W.F. Patton, M.F. Lopez, P. Barry and W.M. Skea, BioTechniques, 12 (1992) 580–585.
[50] M. Chiari, L. D'Alesio, R. Consonni and P.G. Righetti, Electrophoresis, 16 (1995) 1337–1344.
[51] S. Nochumson, U.S. Pat., 4.504.641 and 4.542.200, 1985.
[52] M. Chiari, C. Micheletti, M. Nesi, M. Fazio and P.G. Righetti, Electrophoresis, 15 (1994) 177–186.
[53] M. Chiari, M. Nesi and P.G. Righetti, Electrophoresis, 15 (1994) 616–622.
[54] C. Gelfi, P.G. Righetti, L. Cremonesi and M. Ferrari, Electrophoresis, 15 (1994) 1506–1511.
[55] A. Bossi, P.G. Righetti and M. Chiari, Electrophoresis, 15 (1994) 1112–1117.
[56] A. Bossi, P.G. Righetti, G. Vecchio and S. Severinsen, Electrophoresis, 15 (1994) 1535–1540.
[57] M. Chiari, M. Nesi, P. Roncada and P.G. Righetti, Electrophoresis, 15 (1994) 953–959.

[58] E. Simò-Alfonso, C. Gelfi, R. Sebastiano, A. Citterio and P.G. Righetti, Electrophoresis, 17 (1996) 723–731.
[59] E. Simò-Alfonso, C. Gelfi, R. Sebastiano, A. Citterio and P.G. Righetti, Electrophoresis, 17 (1996) 732–737.
[60] C. Gelfi, E. Simò-Alfonso, R. Sebastiano, A. Citterio and P.G. Righetti, Electrophoresis 17 (1996) 738–743.
[61] P.G. De Gennes, Scaling Concepts in Polymer Physics, Cornell University Press, Ithaca, NY, 1979.
[62] H.J. Bode, FEBS Lett., 65 (1976) 56–58.
[63] H.J. Bode, Anal. Biochem., 92 (1979) 99–110.
[64] M. Chiari, M. Nesi and P.G. Righetti, J. Chromatogr. A, 652 (1993) 31–40.
[65] Y.C. Bae and D. Soane, J. Chromatogr. A, 652 (1993) 17–22.
[66] J.L. Viovy and T. Duke, Electrophoresis, 14 (1993) 322–329.
[67] P.G. Righetti and C. Gelfi, in P.G. Righetti (Editor), Capillary Electrophoresis in Analytical Biotechnology, CRC Press, Boca Raton, FL, 1996, pp. 431–476.
[68] E. Simò-Alfonso, M. Conti, C. Gelfi and P.G. Righetti, J. Chromatogr. A, 689 (1995) 85–96.
[69] C. Gelfi, A. Orsi, F. Leoncini and P.G. Righetti, J. Chromatogr. A, 689 (1995) 97–105.