UCD 543.545.4
ISSN 1330-9862

review

Electrophoresis on Novel Gel Matrices*

*Branko Kozulić***
Elchrom Scientific AG, Gewerbestrasse 8, 6330 Cham, Switzerland

Received: January 25, 1999
Accepted: February 15, 1999

Summary

In recent years, significant progress has been made in the development of new matrices for gel electrophoresis as well as in our understanding of the mechanism governing the migration of large molecules through gels. This review will describe some of these developments. It will be shown that gels of new generation, called Spreadex gels, possess resolving power that is 2–3 fold higher compared to resolving power of polyacrylamide gels. About 8 cm long Spreadex gels enable the separation of double stranded DNA fragments which differ by a single base pair.

Key words: DNA electrophoresis, new matrices, Spreadex gels

Introduction

Gels for electrophoresis can be prepared by free radical polymerization, by thermally induced gelation, and by a cross-linking reaction taking place simultaneously with gelation. In the first case, gel polymers are formed *in situ* from a monomer, whereas in the other two cases the polymers exist in a solution prior to its gelation. Typical representatives are polyacrylamide gels, agarose gels, and Clearose gels *(1)*, but other gels, based on different starting materials, are also known. Given the large number of existing starting materials, a great variety of gels can be produced by using different combinations of these materials. Regardless of the chemical composition of gel polymers, there are several properties that every gel for electrophoresis should possess. The polymers need to be essentially free of charged groups, the gel must have good optical properties and mechanical stability, and the migrating molecules should not interact with the gel matrix in a way which diminishes resolving power of the gel. The analysis of biological macromolecules, including proteins and nucleic acids, represents the most important application of gel electrophoresis. This review describes certain aspects of gel electrophoresis in view of the past and current practice.

Polyacrylamide gels, introduced in 1962 *(2)*, have been established as the matrix of choice for the analysis of proteins. They can be prepared in different formats and used for electrophoresis in various buffers under denaturing or non-denaturing conditions. Polyacrylamide gradient gels are particularly useful for estimating the size of proteins covering a wide size range, and such gels are known to improve size estimation of glycosylated proteins *(3)*. We used polyacrylamide gradient gels in the study of yeast acid phosphatase, a heavily glycosylated protein with pronounced heterogeneity of its carbohydrate part *(4–8)*, and in the development of new methods for cross-linking of glycoproteins *(9–11)*. Two-dimensional electrophoresis, with isoelectric focusing in the first and SDS electrophoresis in the second dimension *(12)*, enables simultaneous analysis of hundreds, or even thousands of proteins. Currently, two-dimensional electrophoresis plays a key role in proteomics, that is, in establishing the function of proteins in the organisms whose genomes are sequenced. When a native 3–30 % polyacrylamide gradient gel is run in the first dimension, followed by second dimension SDS electrophoresis and Western blotting, it is possible to determine the number of subunits of an enzyme present in a crude extract *(13, 14)*. Two-dimensional electrophoresis, with peptide mapping in the second dimension, can be used to distinguish various isoenzymes *(15)*.

In contrast to polyacrylamide gels, agarose gels are used mostly for the analysis of nucleic acids rather than

* Dedicated to Professor Pavao Mildner for his 80th birdthday

** Corresponding author: fax ++ 41/41 741 39 66

proteins. Agarose gels are advantageous because they are easy to use and highly porous, allowing electrophoretic migration of large molecules. The biggest advantage of polyacrylamide gels is their high resolving power. Yet, none of these two matrices is ideal. The drawbacks of agarose gels include brittleness, opacity and poor resolution of small molecules, while polyacrylamide gels are unsuitable for analyzing large molecules. Several research groups have studied new gel matrices in order to find those which would avoid the limitations imposed by acrylamide and agarose gels *(1)*. We synthesized an acrylamide type monomer, N-acryloyl-tris(hydroxymethyl)aminomethane (NAT), and successfully used it for gel electrophoresis of proteins and DNA *(16–18)*. This monomer produces gels which are more hydrophilic than acrylamide gels, and which resolve medium size DNA fragments better than do agarose or polyacrylamide gels. Even more hydrophilic gels, based on amino sugar alcohols, were also synthesized and used for electrophoresis *(19–21)*. Another gel type, based on cross-linked linear polysaccharides such as agarose (Clearose gels) was developed *(22)*. Clearose gels are optically clear and elastic, and they provide better resolution of short DNA fragments compared to agarose gels. Electrophoresis of dsDNA is typically performed on gels that are submerged in an electrophoresis buffer. We have discovered a general improvement of gels for submerged gel electrophoresis *(23,24)*, and developed a new apparatus *(25,26)*. Based on experimental results obtained with new gel matrices, augmented by highly reproducible DNA mobility data acquired by using the new apparatus, a novel theoretical model of gel electrophoresis was developed *(27–31)*. Recently, this model served as a starting point in the work on a new generation of gels for DNA electrophoresis *(32)*, called Spreadex gels. These gels represent the first matrix able to provide 2–3 times higher resolving power than standard polyacrylamide gels. Some properties of these new gel matrices are described below, together with an outline of the theoretical background which led to their development.

The Extended Ogston Model

There are currently several models which describe the mechanism of protein and DNA gel electrophoresis (reviewed in reference *31*). According to the extended Ogston model *(33,34)*, electrophoretic mobility of a macromolecule is proportional to the volume fraction of the pores of the gel that the macromolecule can enter. The measured electrophoretic mobility, μ, can be related to the free mobility in solution, μ_o, of a migrating molecule with radius R, as well as to the gel percentage T, total length of the gel fibers, l', and the fiber radius, r:

$$\log \mu = \log \mu_o - \pi l'(r + R)^2 T \qquad /1/$$

or

$$\log \mu = \log \mu_o - K_r \cdot T \qquad /2/$$

where the retardation coefficient, K_r, is defined as

$$K_r = \pi l'(r + R)^2 \qquad /3/$$

The retardation coefficients for various types of macromolecules have been determined by running them in polyacrylamide gels of different percentages. Knowing the retardation coefficients, it is possible to calculate relative changes of electrophoretic mobility after altering the gel concentration. Thus, by increasing the gel concentration by 10 %, for instance from T = 10 % to T = 11 %, the maximal reduction of DNA mobility will be about 50 % *(32)*.

The Reptation Model

The observation that the mobilities of DNA fragments are proportional to the reciprocal of their sizes, led to the development of the reptation (reptation = snake-like movement) model of DNA gel electrophoresis. According to the reptation model *(35–38)*, DNA migration path is constricted by gel fibers so that a DNA molecule migrates through holes which are connected into a »tube«. The molecules orient themselves in the direction of the electric field, and their mobility can be described as:

$$\mu = q/3\xi\,(1/N + E'^2/3) \qquad /4/$$

where μ is the mobility, q is the total net charge, ξ is the frictional resistance for translational motion along the tube, N is the number of tube segments (which is directly related to DNA contour length), and E' is dimensionless reduced electric field. Equation /4/ indicates that mobilities of large DNA molecules, and of smaller DNA molecules at high electric field strengths, will approach a constant value. At that point resolution is lost, which is indeed observed for long DNA molecules in agarose gels *(39)*. The reptation model has been extended to better explain electrophoretic behaviour of long DNA molecules or other electrophoresis data *(40–42)*.

The Door-Corridor (DC) Model

Based in large part on experiments with new gel matrices, I have proposed another model of gel electrophoresis, called the door-corridor (DC) model *(27–31)*. According to this model, during electrophoresis macromolecules do not migrate through existing gel pores, but instead, they push away gel polymers as they migrate. The gels must contain polymers that can be transiently displaced by the migrating molecules. The migration of macromolecules is discontinuous. They move in discrete steps and in each step they pass through one gel layer. The essential feature of the DC model is the notion that there are two ways a macromolecule can pass through a gel layer, *via* a door or *via* a corridor. Doors are defined as openings formed in the region of a gel layer in which the polymer chains have high motional freedom. Formation of a door does not affect the polymers of other gel layers. Corridors are openings formed in the gel layer where the polymers have low motional freedom. To form a corridor, the migrating molecule must deform a gel layer until an opening develops at a place where one or more polymers end or where the polymers are less cross-linked or entangled. The deformation of one gel layer is accompanied by dislocation of some polymers

in at least one layer above and below. If, on the next layer, the migrating macromolecule encounters a similar area, it will again open a corridor. The two corridors may fuse into a single long corridor, spanning several gel layers. To open large corridors, the migrating molecule must be able to sufficiently displace the polymers of different gel layers. The alternative between opening predominantly doors or corridors by a particular migrating macromolecule depends on the balance of two forces. The first force is electrokinetic, and it is exercised on gel layers by all macro-ions moving in the electric field. This force is countered by the resisting force of the polymers in the gel layer, so that the relationship between DNA mobility and the two forces is:

$$\mu = \mu_1 \cdot e^{-Fr/Fe} \qquad /5/$$

where μ_1 is the mobility of unit size of the migrating molecule, F_r is the resisting force of the gel polymers and F_e is the electrokinetic force which the molecules use to create openings. In the DC model, the mobility of a macromolecule is thus proportional to mobility of the smallest segment of that molecule, μ_1, which is equal to one base pair for double stranded DNA.

From the above outline of the three models, it is evident that both, the extended Ogston and reptation models, consider the gel matrix as a static entity which does not undergo any changes while directing the migrating molecules. In contrast, according to the DC model, the gel matrix does undergo local changes through dynamic interactions between the gel polymers and migrating molecules. Due to these interactions the model predicts that profound changes in migration rates are achievable through modification of the arrangement of gel polymers, or by modifying their chemical composition, as such modifications will affect the force that is required to displace the polymers.

New Gels

During evaluation of various gel materials, it was surprisingly observed that some preformed polymers when present during polymerization of a monomer and a cross-linker dramatically change electrophoretic mobility of DNA fragments *(32)*. Within a certain DNA size range, larger DNA molecules migrated up to two orders of magnitude more slowly compared to migration rates of the same molecules in the gel polymerized in the absence of the polymer. That happened at the concentrations of preformed polymers that were well below 10 % of total gel concentration. This finding cannot be accounted for by the extended Ogston model, which predicts that a 10 % change in gel concentration will reduce DNA mobility by at most a factor of two. On many new gels, DNA molecules above certain size could not migrate at all. They remained at the front wall of the sample well. This finding cannot be explained by the reptation models because, if DNA molecules become oriented and move like snakes through the gel, then regardless of their length all molecules should be able to migrate. Experimental results showed that of two DNA fragments differing by 200 bp, for example 400 and 600 bp, the shorter one will migrate as a sharp band while the longer one will stay at the front wall of the sample well. This characteristic property, exclusion limit, was used to describe a series of new precast gels, named Spreadex gels. The composition and concentration of the gel components were optimized to give gels with exclusion limit of 300, 400, 500, 600, 800 and 1200 bp.

The selective retardation of larger DNA molecules on Spreadex gels results in increased spacing between adjacent bands, that is, selectivity of the gel matrix is enhanced. Since DNA bands remain sharp, resolving power of the gel is increased. Due to this increase of resolving power, short gels are sufficient for demanding separations. For example just a 4 cm long Spreadex EL 300 gel is sufficient for resolving DNA fragments that differ by 4 bp (Fig. 1).

Spreadex gels can be prepared with many combinations of the three essential gel components, including a monomer, a cross-linker and a preformed polymer *(32)*. However, gels with improved resolving power are obtained only within a narrow range of relative concentrations of these three components. Outside of an optimal range, the resolving power often becomes significantly worse. The correct range is dependent on the nature of

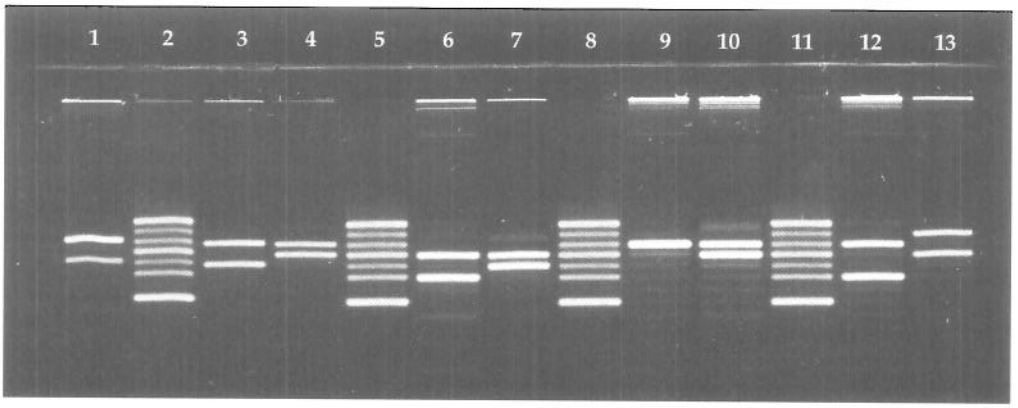

Fig. 1. Electrophoresis of microsatellite DNA fragments derived from human LPL locus on a precast Spreadex EL 300 gel. The gel was run at 10 V/cm for 70 min at 55 °C using the SEA 2000 submarine electrophoresis system (Elchrom Scientific). The LPL locus contains 4 bp repeats. Samples were obtained by PCR using nine different human genomic DNAs. The allelic ladder is in lanes 2, 5, 8, and 11. The fragments contain from 105 to 133 bp. All bands are resolved on less than 4 cm gel length. The gel was stained with SYBR Green I (Molecular Probes)

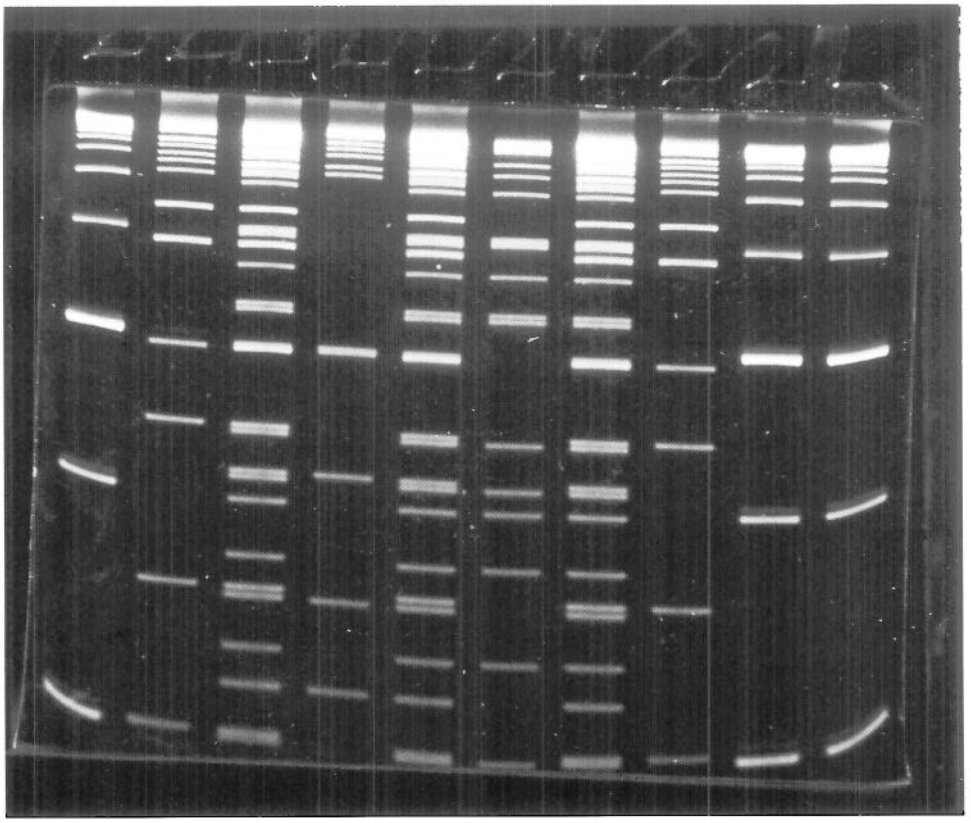

Fig. 2. Electrophoresis of various DNA markers on a 11 % acrylamide-Bis (29:1) gel supplemented with 1X Spreadex Polymer NAB (Elchrom Scientific). The 1 mm thick gel was polymerized in 60 mM TAE buffer using TEMED and ammonium persulfate. The polymerizing solution was injected into a gel cassette using a syringe, followed immediately by overlaying with 50 % methanol in water to get a smooth gel surface. After about 15 min, the unpolymerized solution was removed, the gel surface rinsed with 4 % acrylamide-Bis solution, and then the same 4 % solution polymerized with a comb in place. It is possible to omit the preparation of this second low percentage gel, but then DNA bands are usually less sharp and straight due to uneven gel polymerization under the comb. The gel was run in Mini Protean II vertical apparatus (BioRad) at 200 V for 100 min in 60 mM TAE. Buffer temperature in the cathode compartment increased from room temperature to about 50 °C. The lanes contain: 1, 9, 10 – 25 bp ladder (Gibco BRL), 2, 8 – pBR322/MspI, 3, 5, 7 – M3 marker (Elchrom), 4 – pBR322/Hha I, and 6 – pBR322/Hae III. The M3 marker is a mixture of the three pBR322 digests. In lanes with the 25 bp marker, the band at the bottom contains 75 bp. Four pairs of fragments differing by 1 bp are resolved, including 89 and 90, 103 and 104, 109 and 110, and 131 and 132 bp.

the monomer, the cross-linker and the polymer (32). Recently, we have optimized a Spreadex polymer with the aim of improving the resolving power of common acrylamide-Bis (29:1) gels. The polymer will be commercially available as a 10X stock solution. The polymer is added to acrylamide-Bis solutions of a total monomer concentration between 6 and 12 %, followed by polymerization using TEMED and ammonium persulfate. The resulting gels possess about 3-fold higher resolving power. Thanks to this increase of resolving power, 1 bp differences can now be resolved on standard Mini gels which are only about 8 cm long (Fig. 2). Thus, in the M3 marker shown in lanes 3, 5 and 7, four pairs of bands that differ by 1 bp are resolved (89 and 90, 103 and 104, 109 and 110, and 131 and 132 bp), whilst one (123 and 124 bp) is not.

We should now consider the mechanism by which this increase of resolving power is achieved. The gels of enhanced selectivity are in most cases more opaque than corresponding gels that are made without the third component, the preformed polymer. An increase of scattering of visible light is associated with formation of polymer aggregates, or bundles, whose size corresponds to that of the wavelength of visible light, which is from about 400 nm to 800 nm. Based on a large number of experimental data (*32*), it is hypothesized that these aggregates contain gel polymers which are enriched in the cross-linker, and that the preformed polymers reside inside the aggregates. The strong reduction of DNA migration rate can be rationalized by increased friction between gel polymers. If DNA molecules displace gel polymers during gel electrophoresis, as proposed by the DC model, then any increase of friction between gel polymers will slow down the movement of DNA. Small DNA fragments need to displace only a few polymers. The large ones push away many, in order to form openings through which they migrate. When a gel contains polymer aggregates, then large DNA molecules displace these aggregates. If the aggregates are linked together by another polymer, then the migrating DNA may not be able to displace the aggregates due to a high friction inside the aggregates between gel fibers and preformed polymers linking the aggregates. It is proposed that owing to an increased level of cross-linking, and due to a high local concentration of the gel polymers, inside the aggregates the preformed polymers have little free space. In addition, along their length there may be loops

and hairpin structures, intertwined with gel polymers. Displacement of two aggregates requires that the added polymers are pulled out from one of them. If that takes a larger force than a migrating DNA possesses, then the DNA will not migrate at all. The new gels are thus able to give adequate resistance to the migrating DNA. In effect, the resisting force of the gel polymers is sufficient to prevent the formation of corridors in gel layers.

In addition to a high resolving power, the new gels are able to reduce anomalous sequence-dependent mobilities of DNA fragments *(32)*. This is also evident from the gel shown in Fig. 2, where all fragments derived from pBR322 migrated as expected from their size. This plasmid is known to have two regions which contain sequences that cause significant anomalous migration on regular polyacrylamide gels *(43)*. The ability to perform demanding separations on short gels is beneficial not only because short gels are easier to cast and handle, but also because lower amounts of reagents are needed for gel preparation and for detecting separated bands. Furthermore, bands are always sharper on short gels than on long ones.

In conclusion, future advances of gel electrophoresis will certainly happen with our better theoretical understanding of the mechanism underlying the movement of macromolecules through gels, as well as with the development of new gel matrices and instruments. Thanks to its high resolving power, the requirement for only minute amounts of sample, high speed, throughput, reproducibility, and low cost, gel electrophoresis will remain the key method in the area of protein and DNA analysis.

References

1. B. Kozulić: *Molecular Biology: Current Innovations and Future Trends*, A. M Griffin, H. G. Griffin (Eds.), Horizon Scientific Press, Wymondham, UK (1995) pp. 51–68.
2. S. Raymond, M. Nakamichi, *Anal. Biochem. 3* (1962) 23–30.
3. P. Lambin, J. M. Fine, *Anal. Biochem. 98* (1979) 160–168.
4. S. Barbarić, B. Kozulić, B. Ries, P. Mildner, *Biochem. Biophys. Res. Commun. 95* (1980) 404–409.
5. B. Kozulić, S. Barbarić, B. Ries, P. Mildner, *Period. Biol. 82* (1980) 357–361.
6. S. Barbarić, B. Kozulić, B. Ries, P. Mildner, *Biochem. Int. 2* (1981) 381–388.
7. S. Barbarić, B. Kozulić, B. Ries, P. Mildner, *J. Biol. Chem. 259* (1984) 878–883.
8. B. Kozulić, S. Barbarić, B. Ries, P. Mildner, *Biochem. Biophys. Res. Commun. 122* (1984) 1083–1090.
9. B. Kozulić, I. Leustek, B. Pavlović, P. Mildner, S. Barbarić, *Appl. Biochem. Biotech. 15* (1987) 265–278.
10. U. Heimgartner, B. Kozulić, K. Mosbach, *Biochem. J. 267* (1990) 585–591.
11. S. Barbarić, B. Kozulić, *US Patent 5,258,501* (1993).
12. J. E. Celis, R. Bravo (Eds.): *Two-Dimensional Gel Electrophoresis of Proteins: Methods and Applications*, Academic Press, New York (1983).
13. B. Kozulić, O. Käppeli, F. Meussdoerffer, A. Fiechter, *Eur. J. Biochem. 168* (1987) 245–250.
14. B. Kozulić, K. Mosbach, F. Meussdoerffer, *Biochem. J. 253* (1988) 845–849.
15. M. S. A. Leisola, B. Kozulić, F. Meussdoerffer, A. Fiechter, *J. Biol. Chem. 262* (1987) 419–424.
16. M. Kozulić, B. Kozulić, K. Mosbach, *Anal. Biochem. 126* (1987) 506–512.
17. B. Kozulić, K. Mosbach, M. Pietrzak, *Anal. Biochem. 170* (1988) 478–484.
18. B. Kozulić, K. Mosbach, *US Patent 5,319,046* (1994).
19. B. Kozulić, U. Heimgartner, *US Patent 5,185,466* (1993).
20. B. Kozulić, *US Patent 5,202,007* (1993).
21. B. Kozulić, U. Heimgartner: *Advances in Molecular Cell Biology, Vol. 15B* E. Edward Bittar (Ed.), JAI Press, Hampton Hill (1996) pp. 593–604.
22. B. Kozulić, *US Patent 5,541,255* (1996).
23. B. Kozulić, *Anal. Biochem. 216* (1994) 253–261.
24. B. Kozulić, *US Patent 5,458,760* (1995).
25. B. Kozulić, U. Heimgartner, *Anal. Biochem. 198* (1991) 256–262.
26. B. Kozulić, U. Heimgartner, *US Patent 5,259,943* (1993).
27. B. Kozulić, *Appl. Theor. Electrophoresis, 4* (1994) 117–123.
28. B. Kozulić, *Appl. Theor. Electrophoresis, 4* (1994) 125–136.
29. B. Kozulić, *Appl. Theor. Electrophoresis, 4* (1994) 137–148.
30. B. Kozulić, *Appl. Theor. Electrophoresis, 4* (1994) 149–159.
31. B. Kozulić, *Anal. Biochem. 231* (1995) 1–12.
32. B. Kozulić, *US Patent 5,840,877* (1998).
33. D. Rodbard, A. Chrambach, *Proc. Natl. Acad. Sci. 65* (1970) 970–977.
34. D. Tietz, *Adv. Electrophoresis, 2* (1988) 109–170.
35. L. S. Lerman, H. L. Frisch, *Biopolymers, 21* (1982) 995–997.
36. O. J. Lumpkin, B. H. Zimm, *Biopolymers, 21* (1982) 2315–2316.
37. O. J. Lumpkin, P. Dejardin, B. H. Zimm, *Biopolymers, 24* (1985) 1573–1593.
38. G. W. Slater, J. Noolandi, *Biopolymers, 25* (1986) 431–454.
39. E. M. Southern, *Anal. Biochem. 100* (1979) 319–313.
40. B. H. Zimm, *Phys. Rev. Lett. 61* (1988) 2965–2968.
41. S. Burlatsky, J. Deutch, *Science, 260* (1993) 1782–1784.
42. T. A. J. Duke, J. L. Viovy, *Phys. Rev. Lett. 68* (1992) 542–545.
43. N. C. Stellwagen, *Adv. Electrophoresis, 1* (1987) 179–228.

Novi gelovi za elektroforezu

Sažetak

Posljednjih godina postignut je velik napredak u razvoju novih gelova za elektroforezu, a i u razumijevanju mehanizma putovanja velikih molekula kroz njih. Neka od tih dostignuća opisana su u ovom radu. Prikazani rezultati pokazuju da gelovi nove generacije, pod nazivom gelovi Spreadex, imaju moć razlučivanja koja je 2–3 puta veća od moći razlučivanja poliakrilamidnih gelova. Gelovi Spreadex, kad su dugi samo oko 8 cm, mogu razdvojiti dva odsječka DNA što se međusobno razlikuju za samo jedan par baza.