Hydrogels in Electrophoresis: Applications and Advances

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A hydrogel is a solid form of polymer network absorbed in a substantial amount of aqueous solution. In electrophoresis, hydrogels play versatile roles including as support media, sieving matrixes, affinity scaffolds, and compositions of molecularly imprinting polymers. Recently, the study of hydrogels has been advancing with unprecedented speed, and the application of hydrogels in separation science has brought new opportunities and possible breakthroughs. A good understanding about the roles and effects of the material is essential for hydrogel applications. This review summarizes the hydrogels that has been described in various modes of electrophoretic separations, including isoelectric focusing gel electrophoresis (IEFGE), isotachophoresis (ITP), gel electrophoresis and affinity gel electrophoresis (AGE). As microchip electrophoresis (ME) is one of the future trends in electrophoresis, thought provoking studies related to hydrogels in ME are also introduced. Novel hydrogels and methods that improve separation performance, facilitate the experimental operation process, allow for rapid analysis, and promote the integration to microfluidic devices are highlighted.

Keywords Hydrogel, electrophoresis, electrophoretic separations, rapid analysis, microfluidic device

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1 Introduction

A hydrogel is one type of three-dimensional (3D) network formed by the crosslinking of hydrophilic polymers absorbed in a substantial amount of aqueous solution. Hydrogels can be formed from naturally derived and/or synthetic polymers via physical and/or chemical crosslinking. These polymers are synthesized via various gelation mechanisms, such as thermal condensation, self-assembly, ionic gelation, electrostatic interaction, and chemical crosslinking. One of the many fascinating characteristics of hydrogels is their diverse functionality. Depending on the chemical composition of the hydrogels’ polymers, hydrogels can possess tunable physical and chemical properties, release physical or biochemical cues spontaneously or upon external stimuli, allow for spatiotemporal...
control. Furthermore, many synthetic methods, including controlled polymerization, click chemistry, combination of gelation mechanisms, and doping with nanomaterials, provide approaches for controlling their physicochemical properties. Hydrogels have attracted considerable interest for their potential applications in drug delivery, bio mimics in vitro, tissue engineering, and soft sensors and actuators. Hydrogels also play important roles in the field of separation science. Especially, in the field of electrophoretic separation, hydrogels have been intensively used for the analysis of biomolecules, such as nucleic acids, proteins, and polysaccharides. These biomolecules can be separated by electrophoresis using one or more types of separation modes, for example, isoelectric focusing gel electrophoresis (IEFGE), isocaptoelectrophoresis (ITP), gel electrophoresis (including slab gel electrophoresis (SGE), capillary gel electrophoresis (CGE) and microchip gel electrophoresis (MGE)), micellar electrokinetic chromatography (MEKC), affinity gel electrophoresis (AGE), etc. In the various modes of electrophoresis, the hydrogel may play different roles: they are introduced in order to provide a convection-restricted flow environment for improving band profile, a size sieving network for molecular sieving, a solid scaffold for affinity and immune-affinity probes, or serve as the component of molecular imprinted polymers (MIP). With the aim of providing a comprehensive understanding of the hydrogels’ functions in electrophoresis, this work attempts to review the application of hydrogels within the framework of a categorization of their roles in electrophoresis, which is indicated by the titles of each subsection.

Hydrogel-based electrophoresis comes with both opportunities and challenges. Gel electrophoresis has been recognized as a versatile tool with remarkable resolution for the size sieving of biomolecules, assisted by the tunable pore-size and stiff network of the hydrogel. In addition, the integration of electrophoresis in microfluidic devices, i.e. microchip electrophoresis (ME), is one of the trends for the future. The hydrogel material can also realize many possibilities in ME devices, no matter for which separation mode(s) as mentioned above, owing to its properties of easy filling of the low viscous precursor solution, stimuli-responsiveness of the network and regional pattern control. At the same time, practical challenges also exist that impeded the promotion of hydrogel-based electrophoresis. For example, the conventional hydrogel network is brittle and accompanied with a short lifetime for reproducible performance. Although there are replaceable polymer solutions affording high repeatability, the separation resolution is compromised. In microfluidic devices, changing the absorbing solvent of the hydrogel is important for molecular analysis, however it requires developing tricky protocols. Also, the complicated operations in removing the solid network hinder the rapid recycling of columns as well as chip devices. Thus, it is necessary to develop new materials and protocols to resolve these problems. Fortunately, we are witnessing the emergence of several thought-provoking studies, which are promoting the separation performance of electrophoresis and expanding the areas of application for novel hydrogels. In this review, we make a brief introduction of the recent advances in hydrogel-based electrophoretic separation and highlight the emerging methodologies for hydrogel manipulation in electrophoresis.

2 Applications of Hydrogel in Electrophoresis

2.1 Support media

The use of hydrogels in electrophoresis can be traced back to 1949 from the reported literature. The early form of electrophoresis usually involved a bulk of solution as background electrolyte (BGE). To cut down the BGE convection currents and diffusion, the agar gel was employed as a support media and the separated protein remain as sharp zones. Shortly after that, a starch gel and a polyacrylamide (PA) gel were also introduced into electrophoresis. These support media, which are employed for providing a stable aqueous environment are supposed to be analyte-permeable and allow for less analyte adsorption. As the band diffusion and tailing is restricted, the resolving power of electrophoresis was improved in these gels. Till now, the low concentration agarose gel and PA gel play the stabilizer role in isoelectric focusing (IEF), ITP, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The usefulness of hydrogel as support media is gaining prominence in microfluidic devices, as a fluid stabilizer is the key to the success of some tricky missions. For one example, the alteration of high voltage usually causes vortex mixing of the BGE, disturbing the formation of sharp boundaries between electrolytes. In the biochip platform constructed by Marczak et al., a 1 wt% agarose gel was employed to suppress the vortex of BGE, which guaranteed a facile aggregation method for nanoparticles. Lee’s group constructed an electric field gradient focusing miniature device, on which a semi-permeable gel membrane was fabricated between two flow channels to bring out the formation of a gradient conductivity buffer. The proposed poly(ethylene glycol) acrylate/methacrylate (PEG/PEGMA) hydrogel membrane (channel 4 in Fig. 1(b) and (c)) allows the buffer ion to diffuse between the two channels for separation (channel 3 in Fig. 1(b) and (c)) and forming gradient electric field strength (channel 5 Fig. 1(b) and (c), respectively. As the electric current is controlled by the cross-section area of the shaped channel 5, when a voltage is applied, a gradient electric field strength can be produced along the separation channel. In summary, in electrophoresis, hydrogels are able to provide a solid phase that is permeable enough for molecule exchange but also stable enough to restrict the flow rate.

2.2 Molecular sieving

When the pore size of the gel network is tuned to be comparable to the analytes, fractional effects begin to play a role in the size sieving of molecules. Given its intensive application in nucleic acid and protein separation and its contribution to genomics and proteomics study, molecular sieving is probably the most popular and successful application of hydrogel-based electrophoretic separation. Generally, the charged molecules/molecule-molecule complexes, e.g., protein-SDS complexes and molecule-affinity ligands, are electrophoretically driven to migrate through the network. The migration mobility of the charged molecule is a specific parameter used for the molecular size determination, which is related to both the molecular size and mass-to-charge ratio. As smaller size molecules migrate faster and larger ones are more hindered, studying the relationship between the network pore size and the migration mobility is one of the main subjects in the molecular sieving area. There are different forms of size sieving-based gel electrophoresis: SGE, CGE, and microchip gel electrophoresis (MGE). For over four decades, the traditional SGE has been a workhorse tool for the laboratory analysis of proteins and nucleic acids. Currently, SGE still offers the advantage of multilane/high throughput analysis at a low cost over small-channel gel electrophoresis, or CGE and MGE. Besides, the slab gel form is the basis of the multi-dimensional system and
Ultrathin SGE24 and miniature SGE devices have been developed to require a facilitation of operation from sieving matrixes.3 The major drawbacks in SGE is the inferior separation performance due to Joule heating, band broadening, and long analysis time. As shown in Fig. 2, carboxylic multiwall carbon nanotubes (c-MWNT) were introduced into region Z or M of the PA gels for the separation of proteins. The result of native gel electrophoresis showed that in the c-MWNT doping region Z, extra protein bands (bands 1-4) showed up compared to non-doping gels (bands 5-6), and the similar improvement of resolution was found in the c-MWNT doping region M. 28 The nano composition may also act as the nano scale crosslinker to non-doping gels (bands 5-6), and the similar improvement of resolution is compromised because the weak intermolecular interactions among polymers render less rigid networks. In addition, highly viscous polymer solutions, such as linear polyacrylamide, require high pressure (1000 psi) to fill the column. This is impossible for some micro fluidic devices, so high pressures are required for the separation. As well known, a fast speed, high resolution, low sample cost, and automatable electrophoretic separation are realized by capillary electrophoresis (CE). The narrow capillary (inner diameter <300 μm) allows for fast heat dispersion and high separation voltage, giving enhanced separation efficiency and resolution. Given the aforementioned drawbacks of crosslinked hydrogels, such as bubble formation, various polymer solutions have been employed as sieving networks.31 While the replaceable polymer solutions guarantee the reuse of the capillary column with high run-to-run precision, the separation resolution is compromised because the weak intermolecular interactions among polymers render less rigid networks. In addition, highly viscous polymer solutions, such as linear polyacrylamide, require high pressure (1000 psi) to fill the column. This is impossible for some micro fluidic devices, which can only stand moderate pressures (<200 psi).31 To this extent, compared with SGE, the CGE and MGE are more likely to require a facilitation of operation from sieving matrixes.
Thus, the hydrogel with crosslinked network is still appealing for its merits of low viscous precursor solution, as well as high separation power and simplicity to tune the pore size. Plasticizer, such as glycerol, formamide, and other polymers, had been suggested for enhancing the PA gel and agarose gel structures. Methods to prepare the stable and bubble free PA gel columns were developed. In addition, the modified acrylamide crosslinking matrixes were synthesized without any bubble formations.

To solve the dilemma of whether to choose a powerful separation hydrogel or an operation feasible polymer solution, viscosity switchable copolymer gels have been developed. The sieving matrix could be replaced at a low viscosity state, and provide uncompromised separation performance at the high viscosity state. Thermal-responsive polymers/copolymers of hydroxypropylcellulose (HPC) and poly(N-isopropylacrylamide) (PNIPAAm) have been reported and reviewed in the literature. Another reported species is the phospholipid pseudogel assembled by dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) for DNA size sieving. As shown in Fig. 4, the phospholipid pseudogel decreased viscosity dramatically by a small change of temperature (to an extreme, 5 – 6°C), which facilitated the introduction and replacement of the sieving matrix. Nearly single base resolution of short tandem repeats relevant to human identification was accomplished within 30 min.

As introduced above, new types of hydrogels have been developed to facilitate experimental operations; some hydrogels have been developed in an attempt to enhance the separation performance. A tetra-poly(ethylene glycol) (tetra-PEG) hydrogel with ideally homogeneous structure was demonstrated to possess higher mechanical strength than agarose gel and PA gel (Fig. 5). As is shown in Fig. 5(a), two types of tetra-PEG monomers were respectively modified with the amine functional group and NHS-glutarate group and thus can polycondensate with high efficiency. The tetra-PEG hydrogels at stoichiometrically optimized conditions were reckoned to be structurally homogeneous and defect-limited, evidenced by its ability to endure high compression pressure. Studies using tetra-PEG gel as sieving matrix for the DNA separation revealed a high performance separation with high resolution and short analysis time. It was deduced that the high resolution was ascribed to the dense polymer network and a small amount of polymer material. However, the reproducibility of the tetra-PEG gel capillary was not mentioned. One of the hidden reasons for bubble formation was the presence of tears in the gel matrix as the gelling solution shrinks during polymerization.

Especially, for the PA gels, the volume shrinkage reflects at high monomer concentration. Our group proposed a structure-tunable copoly(poly(ethylene glycol) acrylate/poly(ethylene glycol diacrylate) (copoly(PEGA/PEGDA)) hydrogel, which showed negligible volume shrinkage after polymerization. An extended column life-time was demonstrated by using the concentrated copoly(PEGA/PEGDA) gel. The CGE separation...
of the standard DNA ladder demonstrated that the sieving
resolution was higher by using the copoly(PEGA/PEGDA)
hydrogel than the PA gel at high concentration. Further works
are required to seek more robust materials for gel electrophoresis.
These two typical studies emphasize the importance of
controlling the network structure in a high-performance
separation.

The application scope of gel electrophoresis is expanding
notably to the area of nanoparticles determination. A few
studies have demonstrated the gel electrophoresis methods for
the analysis of the size, shape, charge, and the interaction with
biomolecules of nanoparticles. For one example, Hanauer,
et al.45 separated silver nanoparticles coated with a charged
polymer by shape using an agarose gel. The components
extracted from the electrophoretic bands showed distinct spectra
(Fig. 6). The TEM imaging confirmed that the separated
particles differed in both the shape and size. It can be foreseen
that applications for gel electrophoresis are likely to broaden in
the future.

2-3 Affinity/immuno-affinity analysis
Affinity electrophoresis is a useful tool to bind, separate, and
analyze bio-related molecules. The mobility of a molecule
varies according to whether or not they interact with the affinity
ligand. Molecules with a similar size are separated depending
on their affinity activities with the ligand; the kinetic interaction
between the ligands and targets are measurable. Hydrogels
serve as the scaffold of the affinity ligands via molecule-
molecule adsorption, entrapment, covalent attachment or
crosslinking (Fig. 7). When combining the mechanisms of
both selective recognition and size sieving, affinity gel
electrophoresis holds great potential for high-resolution selective
separation.

When designing an affinity hydrogel, the choice of a proper
ligand is indispensable because the separation is based on the
different affinities between molecules and ligands. As one
example, the quantitative analysis of phosphorylation is
important because it is a key approach to study the protein
regulation in life science. Kinoshita, et al. developed a
phosphate affinity electrophoresis method, named Phos-tag
SDS-PAGE, using an alkoxide-bridged dinuclear metal complex
(Fig. 8). With the Phos-tag SDS-PGE, the phosphorylated
proteins of similar molecular size were discriminated depending
on their phosphorylation states. Similar to traditional PAGE,
the Phos-tag-based affinity gel electrophoresis allowed the
collection and quantification of the phosphorylated protein of interest. As another example, for the affinity separation of polysaccharide and glycoproteins, studies revealed that the boron compound is amenable owing to its reversible bonding with cis-diol-containing compounds,54 which can be taken for the affinity separation with polysaccharide and glycoproteins.55–57 In addition, not only are the small molecules employed as ligands, but macromolecules are as well, such as nucleic acids, enzymes, antibodies, antigens, etc.58–60 To name a few examples, synthetic analogues of nucleic acids (nucleic acid probes) are frequently used as ligands for proteins, small molecules and cells.61,62 Here, it is worth to note that for the affinity separation in a capillary, natural oligonucleotides are not suitable as the gene ligand, because anionic ligands result in unavoidable local electroosmotic flow, which diminishes the high resolving power of CE.48

In addition to the applications in molecular separation, the affinity hydrogel also provides an approach of inline sample preparation.63,64 Gattus et al.65 realized the inline reaction of sialic acids (the analyte) in a phospholipid nanogel, in which the neuraminidase enzyme was physically trapped. A zone of the neuraminidase-trapped nanogel was formed at the end of the capillary via temperature control (Fig. 9). The reaction time or incubation was controlled by mixing the samples (3′ and 6′ sialic acid linkages) therein the nanogel section via the back-and-forth field.39 Excitingly, the catalysis activity remained stable for as long as 30 days in the nanogel regardless of the concentration, while the enzyme lost the activity within a few days depending on the concentration in an aqueous solution. This method allowed for quantifying neuraminidase enzyme kinetics and distinguishing the composition sialic acid linkages. Also, the capillary nanogel electrophoresis revealed an inexpensive, rapid, and feasible technology for molecule analysis.

Hydrogels also play important roles in the emerging next generation blotting technology.13,17,66 Herr’s group introduced an on-chip Western blotting protocol, where a single piece of acrylamide gel acts as both a molecular sieving matrix during SGE separation at one step of the workflow and a blotting scaffold at another step (Fig. 10(a)).67,68 The PA gel with 30 – 60 μm thickness was fabricated onto a microscope slide. Here, a pore-size gradient PA gel was fabricated by attenuating the UV dose for gel photopolymerization through a grayscale chrome mask (Fig. 10(a) step 3). To be noted is that, by adjusting the gradient concentration of the PA gel, ultra-short separation length was found within 1 mm (Fig. 10(b)).69 After performing the molecular size sieving, the Western blotting was
carried out on the identical PA gel, which was copolymerized with benzophenone methacrylamide monomer, namely the LAVAgel.70 Basically, the polypeptide backbone of protein covalently bonded with the pendant benzophenone groups on the LAVAgel upon photo stimulation (Fig. 10(c)). As is shown by steps 4 and 5 in Fig. 10(a), after the size sieving process, the PA gel on the microscope slide was treated with UV light to immobilize the proteins. Then the target protein was probed by treating the PA gel with labeled antibody. Compared with the conventional Western blot, the proposed Western blot technology
provided many advantages, including high performance, high speed, high sensitivity, small volume requirement sample, high throughput, and automatibility.\textsuperscript{37}

Based on the on-chip Western blotting protocol and other thought-provoking reports from the same group, we would like to highlight two techniques that are related with the hydrogel. (1) The open microfluidic device\textsuperscript{71-74} would be an alternative approach to avoid the difficulties brought by the gel volume change in a strictly confined environment. For example, when a gel swell-deswells upon solvent exchange or shrinks upon polymerization in a thin capillary, voids would appear that interfere with the analytical result. The open microfluidic device allowed for rapid solvent exchange, which is commonly required for molecular binding and removing or a solvent-induced stimulus. (2) The hydrogels capable of multi-task\textsuperscript{75} offered an in situ molecular analysis as well as an approach to minimize the device volume, which is a promising method for future use.

2.4 Molecularly imprinted polymer

Molecularly imprinted polymers (MIPs) are synthesized with a template molecule and certain functional monomers for construction of the specific affinity to target molecules (Fig. 11). As the selectivity is predicted by the template, which relies on the cavity formation and memorized interactions, the MIP offers an approach to produce an artificial affinity not even found in nature.\textsuperscript{76} The MIP technology has additional merits including the stable structure, smart response, cost effectiveness, etc.\textsuperscript{77} The MIP itself is mostly formed of macromolecule/polymer with crosslinked structures (sometimes inorganic materials\textsuperscript{78}). In other words, it may share the same nature with the hydrogel when absorbed in an aqueous solution. On another aspect, the hydrogel may serve as a solid support for MIPs.\textsuperscript{79,80} Thus, the development of the MIP technology is consistent with that of hydrogels.

Currently, the MIP technology has been applied in separation science, including solid phase extraction (SPE), chromatographic, electrochromatographic, and electrophoretic separations. Compared to chromatography and electrochromatography, the electrophoretic separations using the MIP as an affinity ligand have been reported less frequently in published articles. This might be due to the difficulties in packing MIP particles, making fit, handing against fragility, avoiding bubble formation, and keeping transparency of the detection window, which are all connected together to the use of capillaries.\textsuperscript{81} A search of the key words containing “electrophoresis” and “molecularly imprinted polymer” in the Web of Science found fewer than 40 records. Among them, the MIP technology is more frequently used for off column SPE and CE is implemented for elution analysis.\textsuperscript{82} In-column MIP-SPE coupled with CE has also been reported by a few groups.\textsuperscript{83-86} Regarding affinity CE separation, there are reports using the MIP monolith,\textsuperscript{87} MIP coated open-tube,\textsuperscript{88} MIP dispersed electrolyte,\textsuperscript{89} and MIP hydrogels.\textsuperscript{90,91} In fact, instead of using MIP hydrogels, when MIP-based separation is implemented, the monolith column, porous structure column, and open tube are optional, in which cases the MIP structures can be firmly fixed on a solid surface. This forms a contrast to the prosperity of the MIP-hydrogel or MIP-nanogel, both of which have shown successful applications in immunoassay, drug delivery, bioimaging, biomimetic medicine, etc. Compared with the solid surface imprinted MIPs, the hydrogel imprinted MIPs and nano MIPs have the advantages of high surface-to-volume ratio and high mass ratio of active sites.\textsuperscript{92,93} To promote the MIP-hydrogel in separation science, there may be a need to improve the mechanical property of the hydrogels.

3 Prospective and Conclusion

This paper reviewed the roles of hydrogels in electrophoretic separations. Various modes of electrophoresis were explained by employing the hydrogel for applications related to solid support (IEF, ITP), size sieving (SGE, CGE), scaffold (AGE) or multi-task. It has been found that the advancements of the hydrogel, as well as the new methods to manipulate the hydrogel, are promoting the developments in electrophoresis on the areas of separation resolution, operation feasibility, analysis speed, integration capability, etc. Transitioning electrophoresis from SGE and CE to microfluidic devices is one of the paths in the future. During the past two to three decades, hydrogels have evolved from relatively inert and static materials to those incorporating sophisticated feedback mechanisms,\textsuperscript{94,95} from the macroscale to micro/nano scale,\textsuperscript{96,97} and the next step is to achieve more controlled physicochemical properties,\textsuperscript{98,99} and enhanced functionality.\textsuperscript{100-102} It can be foreseen that more advanced hydrogels will be utilized in electrophoretic separations in the future.

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