Synthesis and Applications of Polyacrylamide Gels Catalyzed by Silver Nitrate

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ABSTRACT: Polyacrylamide gels are widely used as matrices for biomolecular analysis and fractionation, and they are being developed as biomaterials for diverse medical and industrial applications. This study reports silver nitrate as a novel catalyst for the synthesis of polyacrylamide gels from acrylamide and *N*,*N*-methylene bisacrylamide monomers. The conditions were defined for silver-catalyzed, free-radical-induced polymerization, and a suitable buffer system was devised for the electrophoretic resolution of nucleic acids. A silver-staining procedure was modified for these gels, and they were compared with *N*,*N*,*N*′,*N*′-tetramethylethylenediamine-catalyzed gels for sensitivity and gel background. Silver nitrate and ammonium persulfate at final concentrations of 100 and 625 μg/mL, respectively, polymerized the resolving gels within 20 min at room temperature. These gels exhibited antimicrobial properties. The gels with ≥10 μg/mL silver nitrate showed a zone of complete inhibition of *Staphylococcus aureus* growth on a Luria–Bertani agar plate. The silver-catalyzed gels were also suitable as antigen- and drug-delivery devices. Silver, acting as both a catalyst and a microbicidal agent, was better than *N*,*N*,*N*′,*N*′-tetramethylethylenediamine for the synthesis of polyacrylamide gels as drug- and oxygen-delivery devices for topical applications. J Appl Polym Sci 119: 1084–1089, 2011

Key words: biological applications of polymers; biomaterials; catalysts; gels; synthesis

INTRODUCTION

Polyacrylamide gels (PAAGs) are indispensable for the electrophoretic analysis and fractionation of biomolecules, and they are increasingly being developed as biomaterials for widespread medical and industrial applications.[1–3] PAAGs were first introduced during the early 1960s for the electrophoretic analysis of biomolecules, and various catalysts have since been discovered for their synthesis.[2,4,5] The polymerization process is initiated by the generation of free radicals. Acrylamide is crosslinked with *N*,*N*-methylene bisacrylamide (Bis) or other crosslinkers at different concentrations to yield PAAGs with different physicochemical properties. For resolving molecules of different size ranges, gels of different porosities are made by the variation of the concentrations of the monomers. Nucleic acids, proteins, and other macromolecules resolved in PAAGs can be visualized by silver-staining methods,[6–8] and improvements in electrophoretic resolution and silver-staining procedures for one- and two-dimensional gels are continuously being reported.[9–11]

With the finding that silver nitrate ($AgNO_3$) can catalyze the free-radical-induced polymerization of acrylamide and Bis, this study was undertaken to define the conditions for the synthesis of PAAGs with $AgNO_3$ as the catalyst and to determine applications such as the electrophoresis and silver staining of nucleic acids, antimicrobial materials,[12] drug-delivery devices,[13] and antigen delivery for animal immunization.

EXPERIMENTAL

Compositions of the catalysts

Stock solutions of $AgNO_3$ (100 mg/mL, aqueous, w/v; catalog number 15802, Qualigens Fine Chemicals, Bombay, India) and ammonium persulfate (APS; 100 mg/mL, aqueous, w/v; catalog number 144142, SRL, Bombay, India) were made. The final concentrations of $AgNO_3$ and APS were 100 and 625 μg/mL, respectively, for the resolving gel (acrylamide concentration >6%) and 150 and 1000 μg/mL, respectively, for the stacking gel (acrylamide concentration = 4%). For comparison, two traditional catalysts—

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TABLE I
Buffers and Reagents for the Synthesis of PAAGs with $AgNO_3$ as the Catalyst

Serial no.	Stock solution/reagent	Resolving gel (10%)	Stacking gel (4.0%)	EB
1	2*M* Tris	1.0 mL (100 m*M*)	125 μL (50 m*M*)	12.5 mL (25 m*M*)
2	0.5*M* boric acid	2.0 mL (50 m*M*)	1.0 mL (100 m*M*)	50.0 mL (25 m*M*)
3	0.5*M* EDTA·Na_2	40 μL (1 m*M*)	10 μL (1 m*M*)	2.0 mL (1 m*M*)
4	2% sodium thiosulfate (w/v, aqueous)	40 μL (0.004%)	5 μL (0.002%)	2.0 mL (0.004%)
5	30% acrylamide/Bis	6.670 mL (10.0%)	0.670 mL (4.0%)	—
6	Deionized water (MilliQ quality)	20.0 mL	5.0 mL	1000 mL
7	100 mg/mL $AgNO_3$ (w/v, aqueous)	20 μL (100 μg/mL)	7.5 μL (150 μg/mL)	—
8	100 mg/mL APS (w/v, aqueous)	125 μL (625 μg/mL)	50 μL (1 mg/mL)	—

N,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED; catalog number T-9281, Sigma Chemical Co, St. Louis, MO) and APS (100 mg/mL, aqueous w/v)—were used at final concentrations of 0.075% for the resolving gel and 0.1% for the stacking gel. All chemicals were analytical-grade.

Composition of the buffer system

The tris-borate-EDTA-sodium thiosulphate (TBE-sts) resolving gel buffer was composed of 100 m*M* tris(hydroxymethyl)aminomethane (Tris; catalog number T-6060, Sigma Chemical), 50 m*M* borate (boric acid, catalog number 12005, Qualigens Fine Chemicals), and 1 m*M* ethylene diamine tetraacetic acid (EDTA; EDTA·Na_2, catalog number E-5513, Sigma Chemical) with 40 μg/mL sodium thiosulfate (catalog number 30236F, Sarabhai BDH, Bombay, India). The TBE-sts stacking gel buffer was composed of 50 m*M* Tris, 100 m*M* borate, and 1 m*M* EDTA with 20 μg/mL sodium thiosulfate. The TBE-sts electrode buffer (EB) was composed of 25 m*M* Tris, 25 m*M* borate, and 1 m*M* EDTA with 40 μg/mL sodium thiosulfate. A 6× sample loading dye (6× SLD) solution was composed of 50 m*M* Tris, 100 m*M* borate, 6 m*M* EDTA, 50% glycerol, and 0.01% bromophenol dye. For the traditional Laemmli gels,[14] 375 m*M* Tris/HCl (pH 8.8) as the resolving gel buffer, 125 m*M* Tris/HCl (pH 6.8) as the stacking gel buffer, 25 m*M* Tris/192 m*M* glycine (catalog number 72439, SRL; pH 8.3) as the EB, and a commercially available 6× SLD solution (Fermentas Inc., Hanover, MD) were used.

Synthesis of the PAAGs

The PAAGs were synthesized from monomers: acrylamide (catalog number A3553, Sigma Chemical) and Bis (catalog number M7279, Sigma Chemical). An acrylamide/Bis stock solution (29.2% : 0.8%, aqueous, w/v) was made and stored at the refrigeration temperature for use within a few weeks. For resolving the 100-bp ladder of DNA markers (100–1000 bp), 10% resolving gels were used, and for the stacking gels, the monomer concentration was 4.0%. The compositions of the resolving and stacking gels are presented in Table I. Both the resolving and stacking gels were polymerized at room temperature, regardless of the catalysts and buffer systems employed.

Electrophoresis of nucleic acids

The polyacrylamide slab gel was synthesized and mounted in a vertical slab gel apparatus (Atto Corp., Tokyo, Japan), and the EB was added to the upper and lower buffer tanks. DNA samples of different concentrations (100-bp DNA size ladder; GeneRuler, catalog number SM0241, Fermentas) were mixed 5 : 1 with a 6× SLD and loaded into the wells in the stacking gel, and the DNA bands were resolved by electrophoresis at a constant current of 20 mA.

Silver staining of the gel

The gel containing the resolved DNA bands was silver-stained by a modified form of Bassam et al.'s method.[8] Briefly, the gel after electrophoresis was fixed in 150 mL of 1*M* nitric acid in a clean polyvinyl plastic box (15 cm × 15 cm × 10 cm) for 45 min with intermittent shaking. The gel was rinsed for 2 min three times in deionized MilliQ water (water purification system, Millipore Corporation, Billerica, MA) and then treated with 150 mL of a precooled (<10°C) 0.1% aqueous solution (w/v) of $AgNO_3$ with formaldehyde (150 μL/100 mL solution; catalog number 20113, SD Chemicals, Bombay, India) for 45 min. After being rinsed in MilliQ water for approximately 60 s, the gel was developed in 3% sodium carbonate with formaldehyde (a 150 μL/100 mL solution). Immediately after the appearance of dark brown bands, the reaction was stopped with 5% acetic acid (catalog number 11007, SRL). The stained gel was stored in 40% methanol (catalog number 132977, SRL), 10% acetic acid, and 50% distilled water.

Antimicrobial activity

Silver-impregnated PAAGs, 1.0 mm thick, were made between sterilized glass plate assemblies with $AgNO_3$ at four different concentrations (10, 250,

1250, and 6250 μg/mL). The 6% gel solution was sterilized by passage through a 0.22-μm disposable syringe filter before it was loaded into the gel assembly. The synthesized gel was handled in a Biosafety cabinet (Yorco Pvt. Ltd., New Delhi, India), and circular discs, 11 mm in diameter, were cut from it and placed on a *Staphylococcus aureus* lawn grown for 3.5 h at 37°C on Luria–Bertani agar plates with a diameter of 90 mm. The plates were then incubated overnight at 37°C and observed for inhibition of any growth around the discs (an indication of antimicrobial activity). Discs of PAAGs synthesized with TEMED in the absence of silver were used as controls.

Antigen embedding in the PAAGs for animal immunization

Chicken albumin type VII (ovalbumin; catalog number A7641, Sigma Chemical) was incorporated at the rate of 1.0 mg/100 mg of dry gel mass in an 8% PAAG catalyzed with $AgNO_3$ and APS as described previously. The gel was meshed into fine particles less than 0.2 mm^2 in size and placed into a syringe, and 100 μg of ovalbumin trapped in 10 mg of the gel in a volume of 100 μL was injected subcutaneously into multiple sites on the backs of Swiss albino mice (n = 5). For comparison, 100 μg of ovalbumin in an equal volume of Freund's incomplete adjuvant was made into a water-in-oil emulsion and was injected subcutaneously in mice (n = 5). Similarly, ovalbumin in Freund's complete adjuvant was injected into five mice. Antiserum was collected 21 days after immunization, and anti-ovalbumin immunoglobulin G levels were measured with an indirect enzyme-linked immunosorbent assay. Permission for the use of experimental animals was obtained from the institutional animal ethics committee.

Drug- and oxygen-delivery device with a silver-catalyzed PAAG as the matrix

A drug-delivery device was constructed according to a modified form of the method of Gibbins and Hopman.[13] Briefly, 250 mg of gum acacia (suspended in 250 μL of isopropyl alcohol) was completely rehydrated in 30 mL of deionized water overnight at 37°C. Then, the following ingredients were added in sequence: 10.0 mL of acrylamide/Bis stock (29.2% : 0.8%, aqueous, w/v), 2.5 mL of glycerol, 1.0 mL of 2*M* Tris base, 2.0 mL of 0.5*M* boric acid, 50 μL of 0.5*M* EDTA·Na_2, 50 μL of 2% sodium thiosulfate, 1.0 mL of penicillin, streptomycin, and amphotericin B (100× stock; Gibco, Invitrogen Corporation, Green Island, N.Y.), 50 μL of 1*M* $AgNO_3$, 370 μL of APS (10%, aqueous, w/v), and 1.0 mL of 38.8 mg/mL sodium carbonate. After thorough mixing of the ingredients, the solution was poured into a 90-mm Petri dish and kept undisturbed for polymerization at room temperature. Then, the gel was dehydrated to reduce the moisture to approximately 30% and rehydrated with an equal volume of 30% hydrogen peroxide O/N at room temperature so that foaming could occur. The sheet of the gel foam containing oxygen was cut to size for the topical dressing of infected wounds.

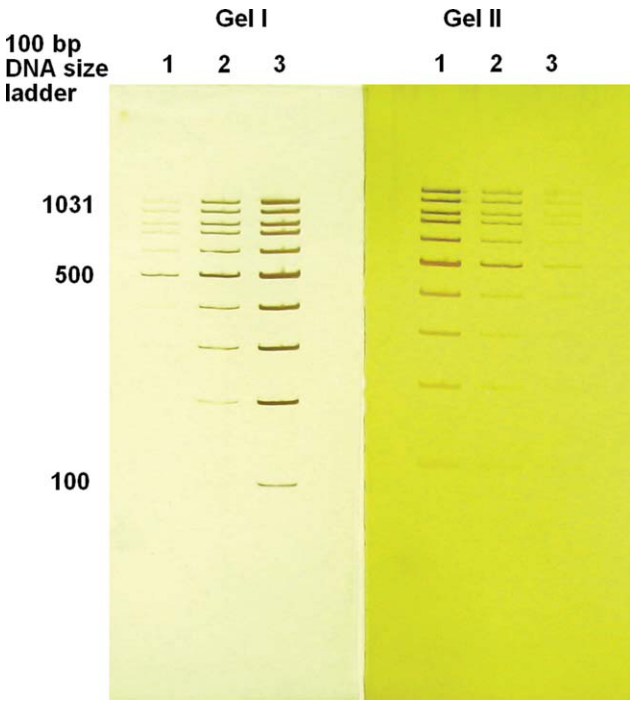

Figure 1 $AgNO_3$/APS-catalyzed PAAGs showing silver staining of the resolved DNA fragments. Various concentrations of 100-bp ladder DNA size markers were resolved in a 10% PAAG. One half of the gel (gel I) was cut and silver-stained with 1*M* nitric acid for fixation (modified from Bassam et al.[8]). The other half (gel II) was stained with 10% acetic acid according to Bassam et al. Gel II had a darker background than gel I. The concentration of the 100-bp ladder DNA size markers (GeneRuler SM0241, Fermentas) increased 4-fold from lane 1 to lane 3 for gel I and decreased 4-fold from lane 1 to lane 3 for gel II. The highest concentrations were 4.0 ng for the 100-bp band, 41 ng for the 500-bp band, and 41.25 ng for the 1031-bp band. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS AND DISCUSSION

$AgNO_3$, in the presence of APS, catalyzed the polymerization of acrylamide and Bis in TBE-sts buffers. Different concentrations of the catalyst and the initiator were required for synthesizing resolving gels and stacking gels of different strengths in approximately 20 min at room temperature. DNA fragments of 100 to more than 1000 bp were resolved in the 10% resolving gel in the TBE-sts resolving gel buffer, as shown in Figures 1 and 2.

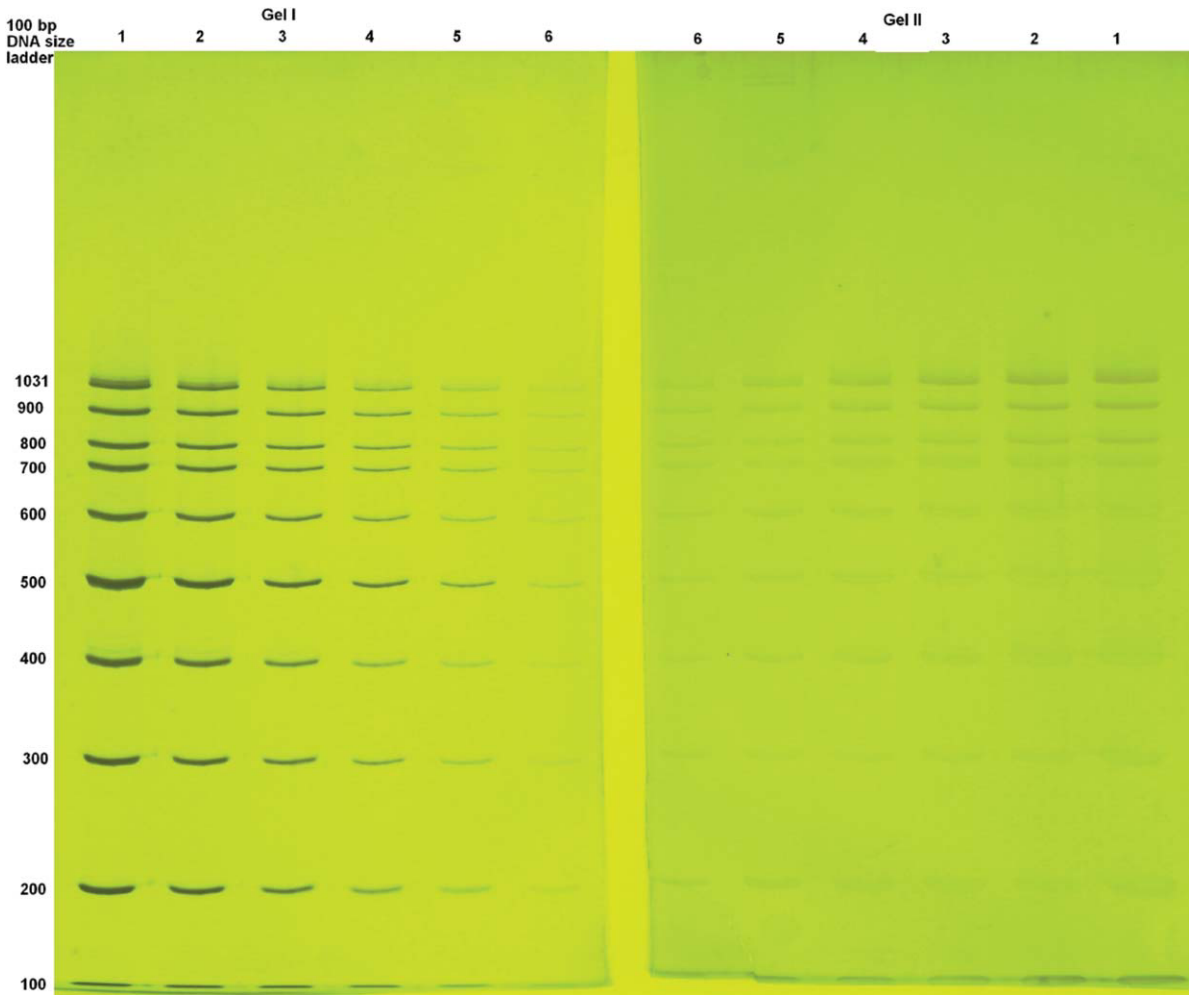

Figure 2 TEMED/APS-catalyzed PAAGs showing silver staining of the resolved DNA fragments. Various concentrations of 100-bp ladder DNA size markers were resolved in a 10% PAAG. One half of the gel (gel I) was cut and silver-stained with 1*M* nitric acid for fixation (modified from Bassam et al.[8]). The other half (gel II) was stained with 10% acetic acid according to Bassam et al.[8] Gel II had a darker background than gel I. The concentration of the 100-bp ladder DNA size markers (GeneRuler SM0241, Fermentas) decreased 2-fold from lane 1 to lane 6 for gels I and II. The highest concentrations were 4.0 ng for the 100-bp band, 41 ng for the 500-bp band, and 41.25 ng for the 1031-bp band (lane 1 for both gels). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Increases in the monomer concentration, incubation temperature, and buffer pH enhanced the rate of polymerization. The gels synthesized with the two different catalysts had similar mechanical strengths and physical appearances. However, yellow discoloration of the silver-catalyzed gels (particularly the stacking gel) occurred upon prolonged storage (>1 day) at room temperature before electrophoresis.

A buffer system developed for the silver-catalyzed gels allowed faster electrophoresis without the resolution being adversely affected. Electrophoresis of the silver-catalyzed gels, carried out in TBE-sts/EB, was complete in nearly 2.5 h at a constant current of 20 mA at room temperature. An initial voltage of 190 V, rising gradually to 224 V after 2.5 h (i.e., by the end of the electrophoretic run), was also observed. The traditional gel in the Tris/glycine EB, however, showed at a constant current of 20 mA a rise in the voltage from 72 V in the beginning to 235 V at the end of electrophoresis; nearly 4 h was needed for the completion of the run at room temperature. No other noticeable difference in the two types of gels was found during electrophoresis. Sodium thiosulfate, present in TBE in all three buffers (i.e., the resolving gel, stacking gel, and EB), was necessary to prevent yellowing of the gel within hours of the polymerization. The incorporation of sodium thiosulfate also improved DNA resolution, probably by forming a complex with silver ions in the gels.

The silver in the PAAGs did not interfere with subsequent silver staining of the resolved DNA bands (Figs. 1 and 2). Gel I in Figures 1 and 2 was fixed in 1*M* nitric acid according to a modification of the method of Bassam et al.,[8] whereas gel II of Figures 1 and 2 was fixed in 10% acetic acid

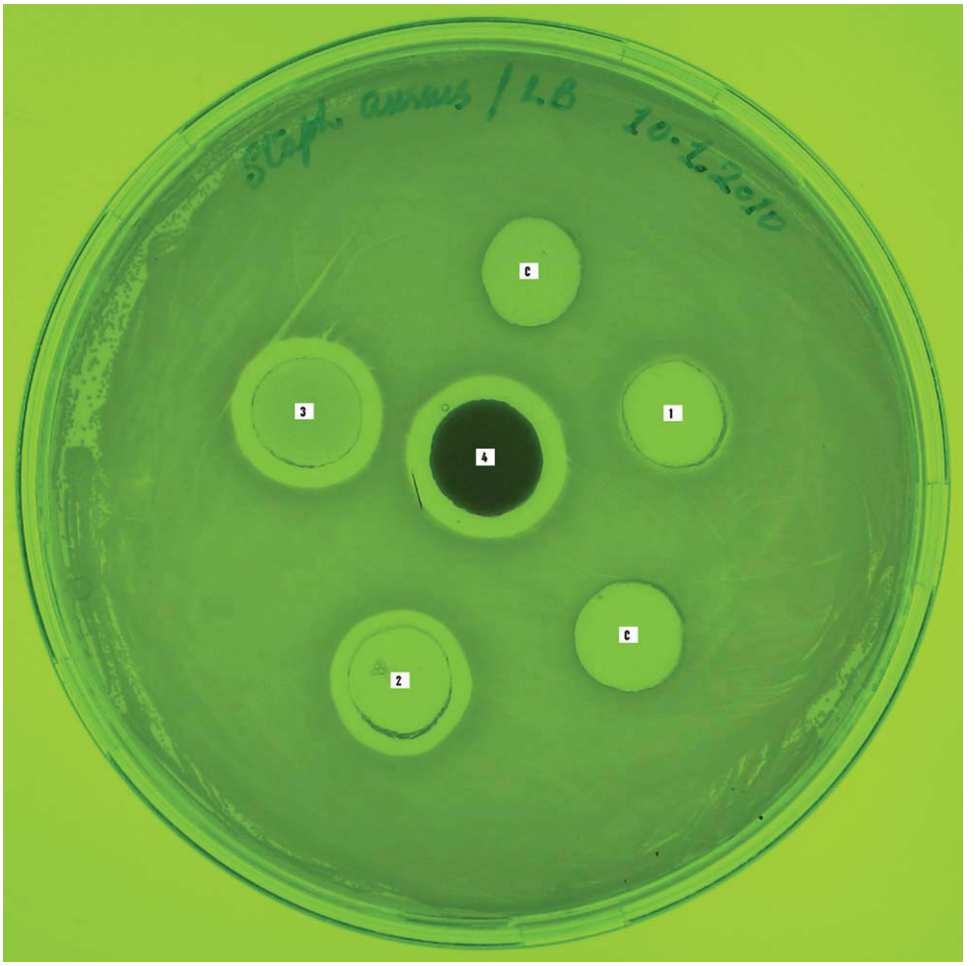

Figure 3 Antimicrobial properties of the silver-catalyzed PAAGs. PAAG discs (each 10 mm in diameter) trapping the catalyst $AgNO_3$ [10 (disc 1), 125 (disc 2), 625 (disc 3), or 6250 μg/mL (disc 4)] were placed onto a lawn of *S. aureus* growth on a Luria–Bertani agar plate. Control discs (C) were PAAGs catalyzed with TEMED. Bacterial growth inhibition zones were measured after 20 h of incubation at 37°C. Bacterial growth was not inhibited by the control discs, whereas inhibition zone diameters of 11.5, 13.6, 14.7, and 15.8 mm were obtained around discs 1, 2, 3, and 4, respectively. The diameter of the inhibitory zone had a positive linear relationship with the logarithm of the concentration of the gel-trapped $AgNO_3$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

according to the method of Bassam et al.[8] Dark brown to black DNA bands developed against an easily controllable transparent background in gel I, whereas golden to dark brown bands developed against a difficult-to-control pale yellowish background in gel II. Negative staining of the DNA bands did not occur in any of these gels. Although methods that result in a transparent background and an absence of negative or hollow bands without the use of a nitric acid fixative exist in the literature,[9,15] the background of the silver-catalyzed gels was definitely improved by nitric acid fixation. The sensitivity of detection was 1–2 pg/mm^2, which was nearly as good as that of the most sensitive method,[8] but only when acetic acid was used as the fixative for the silver-catalyzed gels. Although nitric acid as a fixative improved the background of silver- and TEMED-catalyzed gels, there was still a loss of sensitivity of approximately 4–5 times. The high background problem in TEMED-catalyzed gels was also pointed out by Hochstrasser and Merrill,[4] and the level of sensitivity was improved over the existing methods. Further improvement in sensitivity was achieved by Bassam et al.[8] and Bassam and Gresshoff.[11]

Noticeable differences in the gel background and band coloration were thus found because of the substitution of acetic acid with nitric acid as a fixative. A clear, transparent background was achieved in the latter. The speed of band development was more easily controlled in the silver-catalyzed gels versus the traditional gels. Nitric acid was useful in yet another way. Both silver-catalyzed and traditional silver-stained gels could be destained by a treatment with three half-hour changes of 150 mL of 10% nitric acid at room temperature. The destained gels could be restained without a loss of sensitivity after being washed two times for 10 min in 150 mL of deionized water.

More interestingly, silver-catalyzed gels, exhibiting antimicrobial properties and being used as drug-delivery devices superior to existing ones, could be developed as biomaterials. In this study, silver-catalyzed gel discs with $AgNO_3$ at concentrations between 10 and 6250 μg/mL showed clear zones of inhibition of *S. aureus* growth on a Luria–Bertani plate (Fig. 3). Because both the catalysis and trapping of silver occur in a single step, the method is simpler than others for developing silver-trapped gels with antimicrobial activity.[16]

PAAGs have been trapped with antibiotics, antimycotics, proteins, gases, and so forth for the development of drug-delivery devices and topical dressings for the treatment of burns, skin infections, wounds, and so forth.[13] In this study, silver was substituted for TEMED for the synthesis of a polyacrylamide-based matrix for trapping oxygen and other active agents,[13] and it provided the additional advantage of antimicrobial properties; this would make sterilization of the dressing biomaterial unnecessary. In another experiment, antigen embedded in the silver-catalyzed gel elicited the production of antibody levels detected by an indirect enzyme-linked immunosorbent assay 21 days after a single injection in mice. The antibody levels were comparable to those produced by Freund's incomplete adjuvant antigen. The antigens trapped in these gels are safer than TEMED-catalyzed gels for animal immunization and antigen delivery. Future studies will be aimed at exploring more applications of silver-catalyzed PAAGs as biomaterials, particularly for developing topical dressings and delivery devices for various antigens.

CONCLUSIONS

$AgNO_3$ with APS catalyzes the synthesis of PAAGs. Such gels with a Tris/borate/EDTA/sodium thiosulfate buffer system are useful for the resolution and silver staining of DNA fragments. The silver-catalyzed gels also have antimicrobial properties and are suitable for antigen embedding for animal immunization. Drug- and oxygen-delivering topical dressings can be prepared more conveniently with silver catalysis versus the existing method.

References

1. Chrambach, A.; Rodbard, D. Science 1971, 72, 440.
2. Osterman, L. A. Methods of Protein and Nucleic Acids Research; Springer-Verlag: Berlin, 1984; Vol.1.
3. Yang, T.-H. Recent Pat Mater Sci 2008, 1, 29.
4. Hochstrasser, D. F.; Merrill, C. R. U.S. Pat.5,292,665 (1985).
5. Iwahara, K.; Hirata, M.; Honda, Y.; Watanabe, T.; Kuwahara, M. Biotechnol Lett 2000, 22, 1355.
6. Switzer, R. C.; Merril, C. R.; Shifrin, S. Anal Biochem 1979, 98, 231.
7. Goldman, D.; Merrill, C. R. Electrophoresis 1982, 3, 24.
8. Bassam, B. J.; Anollés, G. C.; Gresshoff, P. M. Anal Biochem 1991, 196, 80.
9. Rabilloud, T. Electrophoresis 1992, 13, 429.
10. Gromova, I.; Celis, J. E. In Cell Biology: A Laboratory Handbook, 3rd ed.; Celis, J. E.; Carter, N.; Hunter, T.; Simons, K.; Small, J. V.; Shotton, D., Eds.; Elsevier Academic: London, 2006; Vol.4.
11. Bassam, B. J.; Gresshoff, P. M. Nat Protocols 2007, 2, 2649.
12. Chopra, I. J Antimicrob Chemother 2007, 59, 587.
13. Gibbins, B. L.; Hopman, L. D. U.S. Pat.7,160,553 (2007).
14. Laemmli, U. K. Nature 1970, 227, 680.
15. Chevallet, M.; Luche, S.; Rabilloud, T. Nat Protocols 2006, 1, 1852.
16. Gibbins, B. L.; Hopman, L. D. U.S. Pat.10215 (2004).