(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
3 February 2005 (03.02.2005)

PCT

(10) International Publication Number
WO 2005/010520 A1

(51) International Patent Classification[7]: G01N 27/447, 27/453

(21) International Application Number: PCT/US2004/022790

(22) International Filing Date: 14 July 2004 (14.07.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/623,480 18 July 2003 (18.07.2003) US

(71) Applicant *(for all designated States except US)*: **BIO-RAD LABORATORIES, INC.** [US/US]; 1000 Alfred Nobel Drive, Hercules, CA 94547 (US).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **PANATTONI, Cory, M.** [US/US]; 316 Creekside Way, Winters, CA 95694 (US).

(74) Agents: **HEINES, M., Henry** et al.; Townsend and Townsend and Crew LLP, Two Embarcadero Center, 8th Floor, San Francisco, CA 94111 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
— *with amended claims and statement*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PRE-CAST ELECTROPHORESIS SLAB GELS FROM SUPPLEMENTED MONOMER SOLUTIONS

(57) Abstract: In pre-cast slab gel cassettes, the formation of pathways in which proteins can migrate between the gel and the walls of the cassette to form shadow bands is avoided by including a nonionic amphiphilic polymer of molecular weight exceeding 100,000 in the monomer solution from which the gel is formed and casting the gel with the polymer included. The nonionic amphiphilic polymer also prevents the resulting gel from sticking to the walls when the gel is to be removed from the cassette after electrophoresis.

WO 2005/010520 A1

PRE-CAST ELECTROPHORESIS SLAB GELS FROM SUPPLEMENTED MONOMER SOLUTIONS

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] This invention relates to polyacrylamide gels as used in slab gel electrophoresis.

2. Description of the Prior Art

[0002] When electrophoresis is performed in a slab gel, several samples can be analyzed simultaneously in the same gel and the resulting electropherograms can be observed and read visually by identifying the locations of the bands on the gel that correspond to the individual components. Polyacrylamide is a gel material that is widely used in slab gels.

[0003] Slab gels are frequently supplied in pre-cast form in cassettes that typically contain two flat transparent plates with the gel retained between them. The plates may be glass or plastic, one commonly used plastic being a polystyrene-acrylonitrile blend. A difficulty with certain pre-cast polyacrylamide gels is that during storage the gels appear to separate from the cassette plates. This creates a pathway between the gel and one or both of the plates in which the sample can migrate during electrophoresis. This migration causes shadow bands in the electropherogram which obscure the clarity and identification of the parent bands, i.e., those that are formed as a direct result of the electrophoretic separation. Shadow bands occur most frequently in pre-cast gels that have been stored without cooling.

[0004] Another problem encountered with polyacrylamide slab gels is a tendency of the gels to stick or adhere to the plates. This presents a difficulty once the separation is completed and the gel must be removed from the plates for purposes of staining, photographing or other observation, detection or recordation. Attempts to remove a gel that

is sticking to one or both of the plates can result in a damaged gel and a ruined experiment. This problem is especially acute for gels of low concentration and for gels used for isoelectric focusing.

[0005] The polymerization reaction to form polyacrylamide is inhibited when dissolved oxygen is present in the gel-forming liquid at or near the gel plate. This is especially true when the gel plates are plastic, such as polystyrene-acrylonitrile, for example. To prevent this inhibition from occurring, a coating of polyvinylidene chloride or polyvinyl dichloride (PVDC) is often applied to the plates prior to contacting the plates with the polyacrylamide gel material. Unfortunately, these coatings exacerbate the sticking problem when the gel is an isoelectric focusing gel, for example one with a pH ranging from 5 to 8. In addition, electrophoresis images produced both with and without these coatings often contain irregularities that appear to be the result of a separation between the gel and the plate.

SUMMARY OF THE INVENTION

[0006] The present invention resides in the discovery that both the occurrence of shadow bands due to apparent pathways between a polyacrylamide gel and a gel cassette plate and the adherence of the gel to the plate can be prevented by forming the gel from a monomer solution that includes a high molecular weight, nonionic amphiphilic polymer in addition to the monomers. The polymer is added to the solution before the gel is cast, and casting is then performed with the polymer still present.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

[0007] Examples of nonionic amphiphilic polymers that can be used in the practice of this invention are poly(vinyl alcohol), agarose, poly(vinyl pyrrolidone), poly(ethylene glycol), poly(ethylene oxide), poly(propylene glycol), poly(propylene glycol)/poly(ethylene glycol) copolymers, and linear polyacrylamide. These polymers are fully formed prior to being added to the gel-forming solution, are soluble in the gel-forming solution, and do not have sites available for crosslinking reactions. Polymers for use in this invention are those having molecular weights above 100,000, preferably between about 100,000 and about 8,000,000, more preferably between about 100,000 and about 5,000,000, and most preferably between about 100,000 and about 1,000,000. The weight percent of the polymer in the monomer solution can range widely, although lowering the molecular weight tends to permit equivalent

or similar results with higher weight percents of the polymer. In the case of polyvinyl alcohol, for example, a preferred concentration range is from about 0.5% to about 5% by weight of the monomer solution. When poly(ethylene glycol) or poly(ethylene oxide) is used, a preferred concentration is from about 0.01% to about 0.3% by weight. The concentrations and molecular weights of other nonionic amphiphilic polymers are readily determined by routine experimentation and will in many cases be readily apparent to those skilled in the art.

[0008] The gel-forming solution is an aqueous solution of a monomer mixture that is polymerizable, generally by a free-radical reaction, to form polyacrylamide. Any monomer mixture that has been used or is described in the literature as being useful in forming polyacrylamide gels can be used in the practice of this invention. The monomer mixture typically includes acrylamide, a crosslinking agent, and a free radical initiator. Preferred crosslinking agents are bisacrylamides, and a particularly convenient crosslinking agent is N,N′-methylene-bisacrylamide.

[0009] The gel-forming solution will also typically include a free radical initiator system. The most common system used is N,N,N′,N′-tetramethylenediamine (TEMED) in combination with ammonium persulfate. Other systems will be apparent to those skilled in the art. The gel-forming solution can also contain additional components that are known or used in electrophoresis gels for various reasons. Buffering agents are commonly included since electrophoretic separations are typically performed at designated pH values. Density control agents, such as glycerol, are also useful in many systems, particularly when the resolving gel is formed underneath a stacking gel.

[0010] Among those skilled in the use of electrophoresis and the preparation of electrophoresis gels, polyacrylamide gels are characterized by the parameters T and C, which are expressed as percents and defined as follows (in which "bis" denotes the bisacrylamide crosslinker):

$$T = \frac{(\text{combined weight of acrylamide and bis in grams})}{(\text{volume of aqueous solution in mL})} \times 100$$

$$C = \frac{(\text{weight of bis})}{(\text{combined weight of acrylamide and bis})} \times 100$$

The values of T and C can vary in the present invention as they do in the use of polyacrylamide gels in general. For the purposes of the present invention, a preferred range of T values is from about 3% to about 30%, and most preferably from about 5% to about 20%. A preferred range of C values of from about 1% to about 10% (corresponding to a

range of weight ratio of acrylamide to bisacrylamide of from about 10:1 to about 100:1), and most preferably from about 2% to about 4% (corresponding to a range of weight ratio of acrylamide to bisacrylamide of from about 25:1 to about 50:1).

[0011] The invention is applicable to gels of uniform concentration as well as gradient gels. The methods for forming both uniform and gradient gels are well known in the art.

[0012] The plates that form the gel cassette are chemically inert, transparent materials, either glass or plastic or both. A wide variety of plastics can be used. The plastics are generally injection moldable plastics, and the selection is limited only by the need for the plastic to be inert to the gel-forming solution, the gel itself, the solutes (typically proteins) in the samples to be analyzed in the cassette, the buffering agents, and any other components that are typically present in the samples. Examples of these plastics are polycarbonate, polystyrene, acrylic polymers, styrene-acrylonitrile copolymer (SAN, NAS), BAREX® acrylonitrile polymers (Barex Resins, Naperville, Illinois, USA), poly(ethylene terephthalate) (PET), poly(ethylene terephthalate glycolate) (PETG), and poly(ethylene naphthalenedicarboxylate) (PEN).

[0013] The following examples are offered for illustrative purposes and are not intended to limit the scope of the invention.

EXAMPLE 1

[0014] This example illustrates the use of poly(ethylene oxide)s of molecular weights 116,000, 205,000, 400,000, and 438,000 in separate experiments as a high molecular weight nonionic amphiphilic polymer gel additive in accordance with the present invention.

[0015] Gradient gels were formed by including the various poly(ethylene oxide)s in the following aqueous solutions (all percents by weight):

Solution A:

acrylamide/N,N′-methylene-bisacrylamide (T = 21%, C = 2.6%)
10% glycerol
0.1% TEMED
0.022% poly(ethylene oxide)

Solution B:

acrylamide/N,N′-methylene-bisacrylamide (T = 6%, C = 2.6%)
0.2% TEMED
0.022% poly(ethylene oxide)

Solution C:

1.125 M tris-HCl (tris(hydroxymethyl)aminomethane hydrochloride), pH 8.6

0.15% ammonium persulfate

[0016] The gels were formed in a cassette consisting of two styrene-acrylonitrile plastic plates defining a gel space measuring 13.4 cm × 8.4 cm × 1 mm. Each gel was formed by first pumping a mixture of Solution B and Solution C at a volume ratio of two-thirds B to one-third C into the cassette from the bottom, to achieve a T = 4% stacking gel solution with a poly(ethylene oxide) concentration of 0.015% by weight. A gradient gel was then formed under the stacking gel by pumping a mixture of Solutions A, B, and C at varying amounts of A and B into the cassette under the 4% gel solution. A ratio of two parts by volume of A plus B to one part by volume of C was maintained while the volume ratio of A to B was varied to produce a T gradient extending from 10.5% to 14%.

[0017] Electrophoretic separations were performed on the gels, utilizing a broad molecular-weight range protein standard from Bio-Rad Laboratories, Inc. (Hercules, California USA), consisting of a selection of nine proteins with molecular weights ranging from 6,500 to 200,000, of which five are resolvable by a typical Tris-HCl gel. The separations were conducted with a voltage of 200 V, using a running buffer containing tris-glycine sodium dodecyl sulfate at approximately 35°C for approximately 55 minutes. Separations under these conditions were performed on gels immediately after casting and also on gels that had been stored for 6 days at 37°C.

[0018] A comparison between the fresh gels without poly(ethylene oxide) and the fresh gels with poly(ethylene oxide) at the various molecular weights revealed that the sharpest protein bands were in the gels containing the poly(ethylene oxide) of 438,000 molecular weight, with the sharpness of the bands increasing as the poly(ethylene oxide) molecular weight increased. Comparisons among the 6-day gels revealed a similar progression, with band sharpness again increasing as the poly(ethylene oxide) molecular weight increased.

EXAMPLE 2

[0019] This example is another illustration of the use of poly(ethylene oxide)s as the gel additive in accordance with the present invention, this time using molecular weights of 511,000, 600,000, 1,000,000, 5,000,000, and 8,000,000.

[0020] Slab gels were prepared as in Example 1, using the higher molecular weight poly(ethylene oxide)s cited in the preceding paragraph, all at a concentration of 0.022 weight

%, with a storage time of 7 days. All other materials, procedures, and conditions were the same.

[0021] A comparison between the fresh gels without poly(ethylene oxide) and the fresh gels with poly(ethylene oxide) at the various molecular weights revealed that the sharpest and straightest protein bands were in the gels containing the poly(ethylene oxide) of 600,000 molecular weight, with the sharpness of the bands decreasing and waviness appearing as the poly(ethylene oxide) molecular weight increased above 600,000. Comparisons among the 7-day gels revealed a similar optimum at 600,000 molecular weight. The 7-day gels with this poly(ethylene oxide) had shorter and lighter trailing regions than those with no poly(ethylene oxide), but the trailing regions darkened as the poly(ethylene oxide) molecular weight increased. With poly(ethylene oxide)s of increasing molecular weights, the resulting bands had an increasing waviness in appearance, possibly due to the increasing viscosity of the monomer solutions. This increasing viscosity may have interfered with the mixing of the monomer and buffer solutions (A and C or B and C).

[0022] The foregoing description is primarily for purposes of illustration. Further modifications, substitutions and variations will be apparent to those skilled in the art and will be included within the scope of the invention.

WHAT IS CLAIMED IS:

1. A method for manufacturing a pre-cast polyacrylamide slab gel for use in slab electrophoresis, said method comprising:

(a) placing a gel-forming liquid mixture inside a gel enclosure defined by a pair of chemically inert, transparent plates separated from each other by fixed distance, said gel-forming mixture comprising an acrylamide monomer, a crosslinking agent, a buffer, and a nonionic amphiphilic polymer having a molecular weight exceeding 100,000, in aqueous solution; and

(b) polymerizing said gel-forming mixture into a gel.

2. A method in accordance with claim **1** in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 8,000,000.

3. A method in accordance with claim **1** in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 5,000,000.

4. A method in accordance with claim **1** in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 1,000,000.

5. A method in accordance with claim **1** in which said nonionic amphiphilic polymer is a member selected from the group consisting of poly(vinyl alcohol), agarose, poly(vinyl pyrrolidone), poly(ethylene glycol), poly(ethylene oxide), poly(propylene glycol), poly(propylene glycol)/ poly(ethylene glycol) copolymers, and linear polyacrylamide.

6. A method in accordance with claim **1** in which said nonionic amphiphilic polymer is poly(vinyl alcohol) at 0.5% to 5% by weight of said aqueous solution.

7. A method in accordance with claim **1** in which said nonionic amphiphilic polymer is poly(ethylene glycol) or poly(ethylene oxide).

8. A method in accordance with claim **7** in which said nonionic amphiphilic polymer is poly(ethylene oxide) at 0.01% to 0.3% by weight of said aqueous solution.

9. A method in accordance with claim **1** in which said plates are glass.

10. A method in accordance with claim **1** in which said plates are a plastic selected from the group consisting of polycarbonate, polystyrene, acrylic polymers, styrene-acrylonitrile copolymer, acrylonitrile polymers, poly(ethylene terephthalate), poly(ethylene terephthalate glycolate), and poly(ethylene naphthalenedicarboxylate).

11. A method in accordance with claim **10** in which said plastic is a polystyrene-acrylonitrile blend.

12. A pre-cast polyacrylamide slab gel for use in slab gel electrophoresis, said pre-cast slab gel comprising:

a pair of chemically inert, transparent plates, and

a polyacrylamide gel cast between said plates, said polyacrylamide gel formed by polymerization of an acrylamide monomer and a crosslinking agent, said polymerization having been performed in an aqueous solution comprising said acrylamide monomer, said crosslinking agent, a buffer, and a nonionic amphiphilic polymer having a molecular weight exceeding 100,000.

13. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 8,000,000.

14. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 5,000,000.

15. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 1,000,000.

16. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said nonionic amphiphilic polymer is a member selected from the group consisting of poly(vinyl alcohol), agarose, poly(vinyl pyrrolidone), poly(ethylene glycol), poly(ethylene oxide), poly(propylene glycol), poly(propylene glycol)/ poly(ethylene glycol) copolymers, and linear polyacrylamide.

17. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said nonionic amphiphilic polymer is poly(vinyl alcohol) at .5% to about 5% by weight of said aqueous solution.

18. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said nonionic amphiphilic polymer is poly(ethylene glycol) or poly(ethylene oxide).

19. A pre-cast polyacrylamide slab gel in accordance with claim **18** in which said nonionic amphiphilic polymer is poly(ethylene oxide) at 0.01% to about 0.3% by weight of said aqueous solution.

20. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said plates are glass.

21. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said plates are a plastic selected from the group consisting of polycarbonate, polystyrene, acrylic polymers, styrene-acrylonitrile copolymer, acrylonitrile polymers, poly(ethylene terephthalate), poly(ethylene terephthalate glycolate), and poly(ethylene naphthalenedicarboxylate).

22. A pre-cast polyacrylamide slab gel in accordance with claim **21** in which said plastic is a polystyrene-acrylonitrile blend.

AMENDED CLAIMS

[received by the International Bureau on 10 November 2004 (10.11.04); original claims 1-12 replaced by amended claims 1-12 (3 pages)]

1. A method for manufacturing a pre-cast polyacrylamide slab gel for use in slab electrophoresis, said method comprising:

(a) placing a gel-forming liquid mixture inside a gel enclosure defined by a pair of chemically inert, transparent plates separated from each other by fixed distance, said gel-forming mixture comprising an acrylamide monomer, a crosslinking agent, a buffer, and a nonionic amphiphilic polymer having a molecular weight exceeding 100,000, in aqueous solution;

(b) polymerizing said gel-forming mixture into a gel; and

(c) storing said gel for at least 5 days prior to use in slab electrophoresis.

2. A method in accordance with claim 1 in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 8,000,000.

3. A method in accordance with claim 1 in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 5,000,000.

4. A method in accordance with claim 1 in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 1,000,000.

5. A method in accordance with claim 1 in which said nonionic amphiphilic polymer is a member selected from the group consisting of poly(vinyl alcohol), agarose, poly(vinyl pyrrolidone), poly(ethylene glycol), poly(ethylene oxide), poly(propylene glycol), poly(propylene glycol)/ poly(ethylene glycol) copolymers, and linear polyacrylamide.

6. A method in accordance with claim 1 in which said nonionic amphiphilic polymer is poly(vinyl alcohol) at 0.5% to 5% by weight of said aqueous solution.

7. A method in accordance with claim 1 in which said nonionic amphiphilic polymer is poly(ethylene glycol) or poly(ethylene oxide).

8. A method in accordance with claim 7 in which said nonionic amphiphilic polymer is poly(ethylene oxide) at 0.01% to 0.3% by weight of said aqueous solution.

9. A method in accordance with claim 1 in which said plates are glass.

10. A method in accordance with claim 1 in which said plates are a plastic selected from the group consisting of polycarbonate, polystyrene, acrylic polymers, styrene-acrylonitrile copolymer, acrylonitrile polymers, poly(ethylene terephthalate), poly(ethylene terephthalate glycolate), and poly(ethylene naphthalenedicarboxylate).

11. A method in accordance with claim 10 in which said plastic is a polystyrene-acrylonitrile blend.

12. A pre-cast polyacrylamide slab gel for use in slab gel electrophoresis, said pre-cast slab gel comprising:

a pair of chemically inert, transparent plates, and

a polyacrylamide gel cast between said plates, said polyacrylamide gel formed by polymerization of an acrylamide monomer and a crosslinking agent, said polymerization having been performed in an aqueous solution comprising said acrylamide monomer, said crosslinking agent, a buffer, and a nonionic amphiphilic polymer having a molecular weight exceeding 100,000, and said gel thus formed having been stored between said plates for at least 5 days prior to use in slab gel electrophoresis.

13. A pre-cast polyacrylamide slab gel in accordance with claim 12 in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 8,000,000.

14. A pre-cast polyacrylamide slab gel in accordance with claim 12 in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 5,000,000.

15. A pre-cast polyacrylamide slab gel in accordance with claim 12 in which said nonionic amphiphilic polymer has a molecular weight between 100,000 and 1,000,000.

16. A pre-cast polyacrylamide slab gel in accordance with claim 12 in which said nonionic amphiphilic polymer is a member selected from the group consisting of poly(vinyl alcohol), agarose, poly(vinyl pyrrolidone), poly(ethylene glycol), poly(ethylene

oxide), poly(propylene glycol), poly(propylene glycol)/ poly(ethylene glycol) copolymers, and linear polyacrylamide.

17. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said nonionic amphiphilic polymer is poly(vinyl alcohol) at .5% to about 5% by weight of said aqueous solution.

18. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said nonionic amphiphilic polymer is poly(ethylene glycol) or poly(ethylene oxide).

19. A pre-cast polyacrylamide slab gel in accordance with claim **18** in which said nonionic amphiphilic polymer is poly(ethylene oxide) at 0.01% to about 0.3% by weight of said aqueous solution.

20. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said plates are glass.

21. A pre-cast polyacrylamide slab gel in accordance with claim **12** in which said plates are a plastic selected from the group consisting of polycarbonate, polystyrene, acrylic polymers, styrene-acrylonitrile copolymer, acrylonitrile polymers, poly(ethylene terephthalate), poly(ethylene terephthalate glycolate), and poly(ethylene naphthalenedicarboxylate).

22. A pre-cast polyacrylamide slab gel in accordance with claim **21** in which said plastic is a polystyrene-acrylonitrile blend.

STATEMENT UNDER ARTICLE 19 (1)

Applicant hereby amends the claims of the above-referenced application per the attached Replacement Sheets and offers the present Statement. This amendment and statement are submitted in response to the International Search Report and Written Opinion mailed to Applicant's attorney from the International Searching Authority on 04 November 2004.

In the amendment, claims 1 and 12 are replaced by amended claims bearing the same numbers. All other claims are unchanged.

This amendment emphasizes a key distinction between the disclosure of each of the two reference patents and the present invention, which is the inclusion of the nonionic amphiphilic polymer in a pre-cast gel, i.e., one that is stored for five days or more before use. The shadow-band problem that Applicant's invention mitigates is one that occurs in pre-cast gels that have been stored for several days. Neither Ogawa (US 4,657,656) nor Moi et al. (US 5,938,906) disclose the preparation or pre-cast gels or the use of gels that have been in storage for any significant period of time prior to use. As a result, neither Ogawa nor Moi et al. encounter the problem of shadow bands occurring in pre-cast gels, i.e., gels that have been in storage for at least several days before use. In each reference, the gels are prepared immediately before use, and the reason in the Ogawa disclosure for the inclusion of the water-soluble polymer is to lessen the brittleness of the gels when they are removed from the glass enclosure in which they are held during electrophoresis. This is not a problem that results from the prolonged storage of gels. The Moi et al. patent is cited for its disclosure of plastic plates rather than glass plates, and again makes no mention of pre-cast gels. In fact, the focus in Moi et al. is on the casting step, which is not a step that would be performed by one using a pre-cast gel.

The fact that the phenomenon that Applicant's invention addresses is specific to pre-cast gels is demonstrated by Applicant's numerous examples, which show the results of experiments performed in pre-cast gels in comparison with the same experiments formed in fresh gels. In each case, the distinction is significant, and the improvement provided by Applicant's invention is demonstrated.

Accordingly, Applicant's invention possesses both novelty and an inventive step relative to each of the documents in the International Search Report.

water-soluble polymer is to lessen the brittleness of the gels when they are removed from the glass enclosure in which they are held during electrophoresis. This is not a problem that results from the prolonged storage of gels. The Moi et al. patent is cited for its disclosure of plastic plates rather than glass plates, and again makes no mention of pre-cast gels. In fact, the focus in Moi et al. is on the casting step, which is not a step that would be performed by one using a pre-cast gel.

The fact that the phenomenon that Applicant's invention addresses is specific to pre-cast gels is demonstrated by Applicant's numerous examples, which show the results of experiments performed in pre-cast gels in comparison with the same experiments formed in fresh gels. In each case, the distinction is significant, and the improvement provided by Applicant's invention is demonstrated.

Accordingly, Applicant's invention possesses both novelty and an inventive step relative to each of the documents in the International Search Report.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US04/22790

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : GO1N 27/447,453
US CL : 204/469,470,466, 615, 616
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 204/469,470,466, 615, 616

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 4,657,656 A (OGAWA) 14 April 1987 (14.04.1987), col. 2, lines 47-56 and col. 3, lines 16-32.	1-9, 12-20 ---------- 10, 11, 21, 22
Y	US 5,938,906 A (MOI et al) 17 August 1999 (17.08.1999), col. 5, lines 8-15 and lines 28-30.	10, 11, 21, 22
A	US 4,963,243 A (OGAWA et al) 16 October 1990 (16.10.1990), see entire document	1-22
A	US 4,737,259 A (OGAWA et al) 12 April 1988 (12.04.1988), see entire document	1-22
A	US 4,699, 705 A (OGAWA et al) 13 October 1987 (13.10.1987), see entire document	1-22

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
25 October 2004 (25.10.2004)

Date of mailing of the international search report
04 NOV 2004

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450
Facsimile No. (703) 305-3230

Authorized officer
Kaj K Olsen
Telephone No. 571-272-1300

Form PCT/ISA/210 (second sheet) (January 2004)