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(54) Title: PREPARATION OF DEFECT-FREE POLYACRYLAMIDE ELECTROPHORESIS GELS IN PLASTIC CASSETTES

(57) Abstract: Irregularities in the pore structures of polyacrylamide gels that are formed in cassettes against plastic walls are re-
duced or eliminated by the inclusion of an oxygen scavenger in the gel-forming solution. Avoidance of the irregularities results in
electropherograms with fewer distortions in the solute bands.

PREPARATION OF DEFECT-FREE POLYACRYLAMIDE ELECTROPHORESIS GELS IN PLASTIC CASSETTES

BACKGROUND OF THE INVENTION

5 1. Field of the Invention

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

2. Description of the Prior Art

[02] Slab gels are particularly useful for electrophoresis in view of their ability to accommodate multiple sample analyses and the ease with which the electropherograms can
10 be observed and read by simply identifying the locations of the various bands on the gels that correspond to the individual components. Polyacrylamide is a gel material that is widely used in slab gels.

[03] Slab gels are frequently supplied in pre-cast form, retained between two flat transparent plates in a cassette. The plates are typically made of plastic, and a difficulty that
15 has been observed is an apparent distortion of the solute bands due to irregularities in the gel pore size near the interface between the gel and the plastic. These irregularities are detrimental to the electrophoretic analysis since the pore size affects the migration of the solute bands, and pore size variations cause distortions of the bands.

[04] Polyacrylamide gels are formed from acrylamide monomers and bis-acrylamide
20 crosslinkers by free radical reactions, and molecular oxygen is known to inhibit free radical formation and thereby to limit the growth of the polyacrylamide chain. The problem is particularly acute with polyacrylamide gels formed in plastic enclosures.

SUMMARY OF THE INVENTION

[05] The present invention resides in the discovery that the interface irregularities of
25 polyacrylamide gels that are precast in plastic gel cassettes can be reduced or eliminated by

the inclusion of an oxygen scavenger in the gel-forming solution from which the gel is cast. The monomer mixture in the solution is polymerized with the scavenger present in the solution, and the result is a pre-cast gel with a substantially uniform pore size throughout. Band resolution in the cassette is then comparable to the band resolution that can be obtained with polyacrylamide gels in glass enclosures.

[06] Further details of the invention and its preferred embodiments are set forth below.

DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS

[07] Oxygen scavengers that can be used in the practice of this invention include many of the materials that have been used or disclosed for use as oxygen scavengers in such applications as boiler operations where they are included for purposes of reducing corrosion. Examples of these materials are sodium sulfite, sodium bisulfite, sodium thiosulfate, sodium lignosulfate, ammonium bisulfite, hydroquinone, diethylhydroxyethanol, diethylhydroxylamine, methylethylketoxime, ascorbic acid, erythorbic acid, and sodium erythorbate. Oxygen scavengers of particular interest in the present application are sodium sulfite, sodium bisulfite, sodium thiosulfate, sodium lignosulfate, and ammonium bisulfite, and of these, sodium sulfite and sodium bisulfite are the most preferred.

[08] The gel-forming solution is an aqueous solution of a monomer mixture that is polymerizable, generally by a free-radical reaction, to form polyacrylamide. Monomer mixtures that have been used or are disclosed in the literature for use 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.

[09] The gel-forming solution will also typically include a free radical initiator system. The most common system used is N,N,N',N'-tetramethylethylenediamine (TEMED) in combination with ammonium persulfate. Other systems will be apparent to those skilled in the art.

[10] 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 5% to about 30%, and most preferably from about 10% 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).

[11] The plastic materials used to form the support plates of the cassettes that will benefit from this invention include a wide variety of plastics. 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), polyethylene terephthalate (PET), polyethylene terephthalate glycolate (PETG), and poly(ethylene naphthalenedicarboxylate) (PEN).

[12] The amount of oxygen scavenger included in the gel-forming solution is not critical to the invention and can be varied over a wide range. Certain plastics will require a greater amount of oxygen scavenger than others since the amount of oxygen retained in the plastic varies among different plastics and the manner in which they are formed. The optimal amount of oxygen scavenger may also vary with the choice of scavenger. In general, however, best results will be obtained with a concentration of oxygen scavenger that is within the range of from about 1 mM to about 30 mM, and preferably from about 3 mM to about 15 mM, in the aqueous gel-forming solution. The amount of oxygen scavenger used may also affect the optimal amounts of the other components. For example, certain oxygen scavengers display catalytic activity toward the free radical reaction, and a lower concentration of free radical initiator can then be used when such scavengers are present.

When a sulfite or bisulfite is used, for example, the concentrations of TEMED and

ammonium persulfate (or other free radical initiator system) can be lower than would otherwise be used.

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

5

EXAMPLE 1

[14] A gel-forming solution was prepared by combining the following components in an aqueous solution in the concentrations indicated:

acrylamide/N,N'-methylene-bisacrylamide (T = 12%, C = 2.67%)

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

10

0.067% TEMED

0.05% ammonium persulfate

6 mM sodium sulfite

15

[15] The acrylamide/bisacrylamide mixture in the solution was allowed to polymerize at room temperature (approximately 20°C) for 30-60 minutes to form a gel ready for electrophoresis.

EXAMPLE 2

[16] The following solutions were prepared for use in forming resolving and stacking gels for a gel cassette constructed with one glass plate and one acrylic plastic plate. All percents are by weight unless otherwise indicated, and all solutions are aqueous solutions.

20

TABLE I

Resolving Gel:	deionized H ₂ O	34.5 mL
	1.5 M Tris-HCl, pH 8.8	25.0 mL
	30% (37.5:1) acrylamide/bisacrylamide	40.0 mL
	10% ammonium persulfate	500 μL
	TEMED	50 μL

Stacking Gel:	deionized H ₂ O	6.2 mL
	1.5 M Tris-HCl, pH 6.8	2.5 mL
	30% (37.5:1) acrylamide/bisacrylamide	1.3 mL
	10% ammonium persulfate	50 μ L
	TEMED	10 μ L

[17] A stock solution of sodium sulfite (1 M aqueous) was also prepared, and added to the resolving and stacking gel solutions in different levels as follows:

- (1) No sodium sulfite added to either gel
- (2) 57.1 μ L of stock solution added to both gel solutions to a final sodium sulfite concentration of 72.02 μ g/mL (0.571 mM) in each solution
- (3) 571.4 μ L of stock solution added to both gel solutions to a final sodium sulfite concentration of 720.2 μ g/mL (5.714 mM) in each solution

[18] The gel solutions were placed in the cassette described above, in a gel space measuring 7 cm \times 8.3 cm \times 1 mm. The gels were allowed to polymerize and were then loaded with an *E. coli* lysate in some bands and a standard protein mixture in others. The standard protein mixture was myosin (200 kD), β -galactosidase (116 kD), phosphorylase B (97.4 kD), bovine serum albumin (66.2 kD), ovalbumin (45 kD), carbonic anhydrase (31 kD), trypsin inhibitor (21.5 kD), and lysozyme (14.5 kD). Upon electrophoresis (SDS-PAGE), distortion of the solute bands (i.e., curving upward toward the outer edges) appeared in the end lanes of the standard protein mixture in all gels but was most pronounced in the gels without sodium sulfite and with only 0.571 mM sodium sulfite. In addition, the bands from both the *E. coli* lysate and the standard protein mixture were less broad and sharper with the 5.714 mM sodium sulfite.

EXAMPLE 3

[19] The following solutions were prepared for use as resolving and stacking gels for a gel cassette formed from two plates of uncoated polystyrene-acrylonitrile copolymer (SAN 880B TYRIL®, The Dow Chemical Company, Midland, Michigan, USA). All percents are by weight unless otherwise indicated, and all solutions are aqueous solutions.

TABLE II

Resolving Gel:	deionized H ₂ O	34.5 mL
	1.5 M Tris-HCl, pH 8.8	25.0 mL
	30% (37.5:1) acrylamide/bisacrylamide	40.0 mL
	10% ammonium persulfate	500 μL
	TEMED	50 μL
	Stacking Gel:	deionized H ₂ O
	1.5 M Tris-HCl, pH 6.8	2.5 mL
	30% (37.5:1) acrylamide/bisacrylamide	1.3 mL
	10% ammonium persulfate	50 μL
	TEMED	10 μL

[20] A stock solution of 1 M aqueous sodium sulfite was also prepared and added to the resolving and stacking gel solutions in different levels as follows:

- (1) No sodium sulfite added to either gel
- 5 (2) 57.1 μL of stock solution added to both gel solutions to a final sodium sulfite concentration of 72.02 μg/mL (0.571 mM) in each solution
- (3) 571.4 μL of stock solution added to both gel solutions to a final sodium sulfite concentration of 720.2 μg/mL (5.714 mM) in each solution

[21] The gel solutions were placed in the all-plastic cassette indicated above, in a gel space measuring 7 cm × 8.3 cm × 1 mm. The gels were allowed to polymerize and were then
10 loaded with the same protein mixtures used in Example 2, in different lanes of the gel. Upon electrophoresis (SDS-PAGE), distortion of the solute bands (i.e., wavy bands) appeared in all lanes of the gels without sodium sulfite and with only 0.571 mM sodium sulfite, while the solute bands in the gel containing 5.714 mM sodium sulfite were noticeably straighter. In
15 addition, the bands with the 5.714 mM sodium sulfite were less broad and sharper.

[22] 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 1. A method for casting a polyacrylamide gel in a plastic gel enclosure,
2 said method comprising
3 (a) forming an aqueous solution of a monomer mixture comprising
4 acrylamide, a crosslinking agent, and an oxygen scavenger which is a member
5 selected from the group consisting of sodium sulfite, sodium bisulfite, sodium
6 thiosulfate, sodium lignosulfate, ammonium bisulfite, hydroquinone,
7 diethylhydroxyethanol, diethylhydroxylamine, methylethylketoxime, ascorbic acid,
8 erythorbic acid, and sodium erythorbate; and
9 (b) polymerizing said monomer mixture in a plastic gel enclosure to form a
10 polyacrylamide gel.
- 1 2. A method in accordance with claim 1 in which said monomer mixture
2 further comprises a free radical initiator.
- 1 3. A method in accordance with claim 1 in which said oxygen scavenger
2 is a member selected from the group consisting of sodium sulfite, sodium bisulfite, sodium
3 thiosulfate, sodium lignosulfate, and ammonium bisulfite.
- 1 4. A method in accordance with claim 1 in which said oxygen scavenger
2 is sodium sulfite.
- 1 5. A method in accordance with claim 1 in which the concentration of
2 said oxygen scavenger in said aqueous solution is from about 1 mM to about 30 mM.
- 1 6. A method in accordance with claim 3 in which the concentration of
2 said oxygen scavenger in said aqueous solution is from about 3 mM to about 15 mM.
- 1 7. A method in accordance with claim 1 in which said plastic gel
2 enclosure is a member selected from the group consisting of polycarbonate, polystyrene,
3 styrene-acrylonitrile copolymer, polyethylene terephthalate, polyethylene terephthalate
4 glycolate, and poly(ethylene naphthalenedicarboxylate).
- 1 8. A method in accordance with claim 1 in which said monomer mixture
2 comprises acrylamide and N,N'-methylene-bisacrylamide in aqueous solution, the total of

3 said acrylamide and N,N'-methylene-bisacrylamide amounting to from about 5 g to about
4 30 g per milliliter of said aqueous solution.

1 **9.** A method in accordance with claim **1** in which said monomer mixture
2 comprises acrylamide and N,N'-methylene-bisacrylamide at a combined concentration of
3 from about 10 g to about 20 g per milliliter of said aqueous solution.

1 **10.** A method in accordance with claim **8** in which the weight ratio of
2 acrylamide to N,N'-methylene-bisacrylamide is from about 10:1 to about 100:1.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/06207

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C08F 120/56, 2/10, 4/40; G01N 27/26
US CL :526/234, 934; 524/916; 525/329.4; 204/470

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. : 526/234, 934; 524/916; 525/329.4; 204/470

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)
EAST; polyacrylamide, gel, sodium sulfite, hydroquinone oxygen scavenger, oxidation inhibitor, mold, enclosure, plate.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,292,665 A (HOCHSTRASSER et al) 08 March 1994, see col. 5, lines 6-9, col. 6, lines 25-46 and col. 9, line 34 to col. 10, line 24.	1-3, 5-7
Y	US 5,753,095 A (ALPENFELS et al) 19 May 1998, see col. 1, line 53 to col. 2, line 4, col. 4, lines 51-60 and examples.	1-7
Y	US 4,940,763 A (FLESHER et al) 10 July 1990, see col. 8, lines 14-16.	1, 3, 4
Y	US 3,755,280 A (SAUNDERS) 28 August 1973, see col. 4, lines 32-33.	1, 3, 4
Y	US 6,110,340 A (LAU et al) 29 August 2000, see col. 4, lines 38-40.	1-7

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

* Special categories of cited documents:	"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
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INTERNATIONAL SEARCH REPORT

International application No.
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,806,434 A (OGAWA) 21 February 1989, see example 1 and table 1 and col 13, lines 35-40.	1-10