Acrylamide Polymerization — A Practical Approach

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Introduction

The unparalleled resolution and flexibility possible with polyacrylamide gel electrophoresis (PAGE) has led to its widespread use for the separation of proteins and nucleic acids. Gel porosity can be varied over a wide range to meet specific separation requirements. Electrophoresis gels and buffers can be chosen to provide separation on the basis of charge, size, or a combination of charge and size.

The key to mastering this powerful technique lies in the polymerization process itself. By understanding the important parameters, and following a few simple guidelines, the novice can become proficient and the experienced user can optimize separations even further.

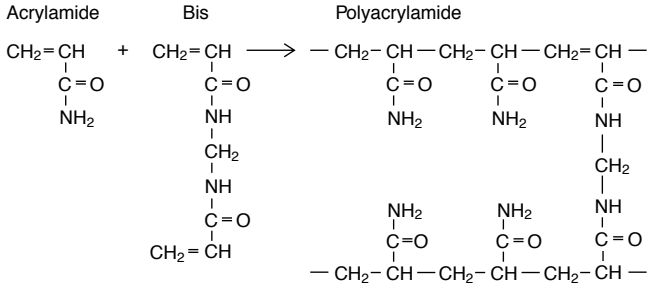
This bulletin takes a practical approach to the preparation of polyacrylamide gels. Its purpose is to provide the information required to achieve reproducible, controllable polymerization. For those users interested only in the "bare essentials," the Polymerization Protocols can be used as a laboratory guide.

Mechanism of Polymerization

Polyacrylamide gels are formed by copolymerization of acrylamide and bis-acrylamide ("bis," N,N'-methylene-bis-acrylamide). The reaction is a vinyl addition polymerization initiated by a free radical-generating system (Chrambach 1985). Polymerization is initiated by ammonium persulfate and TEMED (tetramethylethylenediamine): TEMED accelerates the rate of formation of free radicals from persulfate and these in turn catalyze polymerization. The persulfate free radicals convert acrylamide monomers to free radicals which react with unactivated monomers to begin the polymerization chain reaction (Shi and Jackowski 1998). The elongating polymer chains are randomly crosslinked by bis, resulting in a gel with a characteristic porosity which depends on the polymerization conditions and monomer concentrations.

Riboflavin (or riboflavin-5'-phosphate) may also be used as a source of free radicals, often in combination with TEMED and ammonium persulfate. In the presence of light and oxygen, riboflavin is converted to its *leuco* form, which is active in initiating polymerization. This is usually referred to as photochemical polymerization.

Polyacrylamide Gel Polymerization



Purity of Gel-Forming Reagents

Acrylamide

Gel-forming reagents include the monomers, acrylamide and bis, as well as the initiators, usually ammonium persulfate and TEMED or, occasionally, riboflavin and TEMED. On a molar basis, acrylamide is by far the most abundant component in the monomer solution. As a result, acrylamide may be the primary source of interfering contaminants (Dirksen and Chrambach 1972). Poor-quality acrylamide contains significant amounts of the following contaminants:

1. Acrylic acid — Acrylic acid is the deamidation product of acrylamide. Acrylic acid will copolymerize with acrylamide and bis, thereby conferring ion exchange properties on the resulting gel. This can lead to local pH changes in the gel and cause artifacts such as aberrant relative mobility, precipitation of some proteins and nucleic acids, streaking or smearing of bands, and run-to-run irreproducibility. In acrylamide, acrylic acid should be below 0.001% (w/w). This is determined by direct titration, and supported by both conductivity and pH measurement.

2. Linear polyacrylamide — Contaminants with catalytic properties may cause what appears to be autopolymerization during the production, processing, or storage of marginally pure acrylamide. This results in the presence of linear polyacrylamide in the dry monomer. Linear polyacrylamide will affect polymerization, since it serves as a nucleus for polymerization. The most important effect is the loss of reproducibility in gel porosity and relative mobilities of proteins and nucleic acids. Linear polyacrylamide is detected as water or alcohol insolubles and should be <0.005% (w/w).

3. Ionic contaminants — Ionic contaminants can include both inhibitors and accelerators of polymerization. Aside from acrylic acid, the most notable ionic contaminants are metals such as copper, which can inhibit gel polymerization. Metals can also poison enzymes, alter the relative mobility of metal binding proteins such as calmodulin, and inhibit digestion of electrophoretically purified nucleic acids by restriction and modification enzymes. Ionic contaminants are detected indirectly by their effects on chemical and photochemical polymerization, and by the conductivity of monomer solutions.

bis-Acrylamide

Bis is present in much smaller quantities than acrylamide in monomer solutions. However, improperly purified bis contains some of the same contaminants as acrylamide. These include products of autopolymerization and ionic contaminants, which have the same deleterious effects, and can be detected in the same ways, as the corresponding acrylamide contaminants.

Initiators

Chemical polymerization is initiated by ammonium persulfate, while photochemical polymerization is initiated by riboflavin (or riboflavin-5'-phosphate), or by a combination of riboflavin and ammonium persulfate. Initiation and polymerization are catalyzed by TEMED. Because polymerization is initiated by the generation of free radicals from persulfate or riboflavin, it is not surprising that these compounds are reactive, and prone to oxidation or decomposition. The contaminants of the initiators tend to be the products of their own breakdown as well as other contaminating compounds.

TEMED is subject to oxidation, which causes the gradual loss of catalytic activity. This process is greatly accelerated by contaminating oxidizing agents. TEMED that contains oxidation products is characterized by a yellow color. The practical consequences of the oxidative process are the requirement for greater amounts of TEMED to achieve adequate polymerization, and a gradual loss of TEMED reactivity with time. TEMED is also very hygroscopic and will gradually accumulate water, which will accelerate oxidative decomposition. TEMED with maximum activity and shelf life is obtained by redistillation immediately prior to bottling, resulting in a product that is clear, water free, and greater than 99% pure (14.4 M).

Ammonium persulfate is also very hygroscopic. This property is particularly important, since ammonium persulfate begins to break down almost immediately when dissolved in water. Therefore, the accumulation of water in ammonium persulfate results in a rapid loss of reactivity. This is why ammonium persulfate solutions should be prepared fresh daily. Persulfate is consumed in the polymerization reaction. Excess persulfate can cause oxidation of proteins and nucleic acids. This oxidation problem can be avoided if inhibitor-free gel-forming reagents are used, and ammonium persulfate is used at the recommended levels.

Contaminants in Buffers

Contaminants in buffer reagents (Tris, borate, acetate, glycine, etc.), gel additives (SDS, urea, etc.), and laboratory water can have a profound effect on polymerization. The most common contaminants of these reagents are metals, non-buffer ions, and breakdown products. The most frequent effect of these contaminants is to inhibit polymerization. When polymerization is partially inhibited, the resulting gel will have greater porosity than intended, and molecules will have greater mobilities. Furthermore, control over polymerization reproducibility is compromised.

Initiator Type and Concentration

Initiators are the effectors of polymerization. Of course, the rate of polymerization depends on the concentration of initiators, but more importantly, the properties of the resulting gel also depend on the concentration of initiators. Increasing the concentration of initiators (e.g., ammonium persulfate and TEMED) results in a decrease in the average polymer chain length, an increase in gel turbidity, and a decrease in gel elasticity. In extreme cases, excess initiator can produce a gel solution that does not appear to polymerize at all. This is due to the formation of polymer chains so short that visible gelation does not take place and the polymer stays in solution. The only indication that a reaction has taken place is an increase in viscosity.

Excess ammonium persulfate and TEMED have other effects, including oxidation of sample proteins (especially sulfhydryl-containing compounds) and changes in buffer pH. Excess TEMED can increase buffer pH, react with proteins (Dirksen and Chrambach 1972; Chrambach et al. 1976), and alter the banding pattern (Gelfi and Righetti 1981a). Ammonium persulfate acts as a buffer between pH 8 and 9. Potassium persulfate is recommended instead of ammonium persulfate in weakly buffered basic systems (~pH 9). Excess riboflavin may cause the oxidation of some compounds, especially sulfhydryl-containing compounds (Dirksen and Chrambach 1972), and can denature proteins (Righetti et al. 1981).

Reducing the concentration of initiators results in longer polymer chain lengths, lower turbidity, and greater elasticity. These are desirable properties. However, lower initiator concentrations also mean slower polymerization. If polymerization is too slow, oxygen will begin to enter the monomer solution and inhibit polymerization, resulting in gels which are too porous and mechanically weak. Inhibition will be especially pronounced at surfaces exposed to air, or at the surfaces of combs and spacers, which appear to trap air at their surfaces. The remaining unpolymerized monomer can react with alpha amino, sulfhydryl, and phenolic hydroxyl groups of proteins (Allison et al. 1974; Chrambach et al. 1976; Dirksen and Chrambach 1972).

For discontinuous systems which employ a stacking gel (e.g., Laemmli system), optimal polymerization of the overlaid lower gel (resolving gel) is achieved when visible gelation takes place 15–20 min after the addition of the initiators ammonium persulfate and TEMED (note that polymerization continues long after visible gelation takes place; see Figure 1). For stacking gels and continuous systems (which do not contain stacking gels) — i.e., any gel which is not overlaid — optimal polymerization results when visible gelation takes place in 8–10 min. Higher initiator concentrations and faster polymerization are required in these cases because of the inhibitory effect of atmospheric oxygen associated with the comb. In any case, conversion of monomer to polymer should be greater than 95%. If gelation takes longer than 20 min, the inhibitory effects of atmospheric oxygen will begin to appear.

As a general rule, use the lowest catalyst concentrations that will allow polymerization in the optimal period of time. In the case of ammonium persulfate/TEMED-catalyzed reactions, for example, approximately equimolar concentrations of both catalysts in the range of 1 to 10 mM are recommended.

Riboflavin is often used as an initiator along with TEMED, or with TEMED and ammonium persulfate. The major advantage of riboflavin is that it is active in very low concentrations (~5–10 µg/ml). Thus, when riboflavin is used with TEMED and ammonium persulfate, the total amount of initiator required (sum of the three initiators) is less. Given the possible effects of initiators on buffer pH, riboflavin-based initiator systems are useful for poorly buffered systems such as electrofocusing gels, in which the only buffering components are ampholytes.

Visible gelation takes longer in riboflavin-based initiator systems, usually 30–60 min. Oxygen does not have the dramatic inhibitory effect on riboflavin-based initiator systems that it has on TEMED/ammonium persulfate systems. This is presumably due to the oxygen-scavenging property of riboflavin. As a result, longer gelation time can be tolerated.

In chemical polymerization, visible gelation occurs in 15–20 min and polymerization is essentially complete in 90 min. In photochemical polymerization, however, visible gelation takes 30–60 min and complete polymerization requires up to 8 hr (Righetti et al. 1981). Shorter times lead to more porous and elastic gels, increased risk of protein modification, and pore size irreproducibility.

Temperature

Temperature control is critical for reproducibility of acrylamide polymerization. Temperature has a direct effect on the rate of gel polymerization; the polymerization reaction is also exothermic. Consequently, the generated heat drives the reaction more quickly. Thus, gelation usually occurs very rapidly once polymerization begins.

Temperature also affects the properties of the gel (Chen and Chrambach 1979). For example, polymerization at 0–4°C results in turbid, porous, inelastic gels, and reproducibility is difficult to achieve. These properties may be due to increased hydrogen bonding of monomer at low temperatures. Gels polymerized at 25°C are more transparent, less porous, and more elastic. However, if the polymerization temperature is too high, short polymer chains are formed and the gels are inelastic. This is thought to be due to increased polymer chain termination at higher temperatures.

A temperature of 23–25°C is optimal (as well as most convenient) for polymerization. It is important that the monomer solution and the gel mold (e.g., glass plates or tubes) be at the optimal temperature when the gel is poured. Furthermore, reproducibility is dependent on using the same temperature each time gels are poured.

Since monomer solutions are usually stored at 4°C along with buffer concentrates, it is important to allow the monomer gel solution, once prepared, to equilibrate to room temperature before being evacuated (if cold solutions are placed under vacuum they tend to stay cold).

Oxygen

The formation of polyacrylamide gels proceeds via free radical polymerization. The reaction is therefore inhibited by any element or compound that serves as a free radical trap (Chrambach 1985). Oxygen is such an inhibitor. Oxygen, present in the air, dissolved in gel solutions, or adsorbed to the surfaces of plastic, rubber, etc., will inhibit, and in extreme cases prevent, acrylamide polymerization. Proper degassing is critical for reproducibility. Therefore, one of the most important steps in the preparation of polyacrylamide gels is the evacuation, or “degassing” of gel solutions immediately prior to pouring the gel. This is done by placing the flask of gel solution in a vacuum chamber or under a strong aspirator. In some cases, a vacuum pump may be required.

Buffer stock solutions and monomer stock solutions are usually stored at 4°C. Cold solutions have a greater capacity for dissolved oxygen. The process of degassing is faster and more complete if the gel solution is brought to room temperature (23–25°C), before degassing begins. Furthermore, if a cold gel solution is placed under vacuum, the process of evacuation tends to keep the solution cold. Pouring a gel with a cold solution will have a substantial negative effect on the rate of polymerization and on the quality of the resulting gel.

Polymerization in which riboflavin is used as one of the initiators calls for degassing. The conversion of riboflavin from the *flavo* to the *leuco* form (the species active in initiation) actually requires a small amount of oxygen (Gordon 1973).

This explains why polymerization initiated primarily by riboflavin can be completely blocked by exhaustive degassing. However, oxygen in excess of that needed to convert riboflavin to the active form will inhibit polymer chain elongation, as it does in reactions initiated only by ammonium persulfate and TEMED. Thus, while degassing is still important for limiting inhibition, it must not be so extensive that it prevents conversion of riboflavin to the active form. For polymerization initiated by riboflavin/TEMED, or riboflavin/TEMED/ammonium persulfate systems, degassing should not exceed 5 min.

A consequence of the interaction of riboflavin with oxygen is that riboflavin seems to act as an oxygen scavenger. This is supported by the observation that the addition of riboflavin (5 µg/ml) to stacking gel solutions containing ammonium persulfate/TEMED initiators results in cleaner, more uniform polymerization at gel surfaces exposed to oxygen (such as combs). The same effect could likely be achieved by more thorough degassing of solutions without riboflavin.

Whether using chemical polymerization (ammonium persulfate/TEMED) or photochemical polymerization (riboflavin/TEMED or riboflavin/TEMED/ammonium persulfate initiators), reproducible gel quality and separation characteristics require careful attention to gel solution temperature before degassing, and to degassing time, temperature, and vacuum. These parameters should be kept constant every time gels are prepared.

pH

The majority of electrophoresis systems are buffered at neutral or basic pH, at which the common initiators, ammonium persulfate, TEMED, and riboflavin, are effective. Riboflavin is the better choice for polymerization at low pH (Shi and Jackowski 1998); however, at low pH, TEMED may become protonated. This can result in slower polymerization, since the free base form of TEMED is required for initiation. For acidic buffer systems, alternative initiator systems are sometimes used (Andrews 1990).

Alternative Crosslinkers

PDA (piperazine di-acrylamide), a crosslinking agent that can be substituted for bis in polyacrylamide gels, offers several advantages for electrophoresis. These include reduced background for silver staining, increased gel strength, and higher-resolution gels. PDA can be substituted for bis on a weight basis without changing polymerization protocols.

Crosslinkers other than bis and PDA may be used for specialized purposes, the most common of which is gel solubilization during post-electrophoresis recovery of proteins or nucleic acids. These crosslinkers include DATD (diallyl-tartardiamide), DHEBA (dihydroxyethylene-bis-acrylamide), and BAC (bis-acrylylcystamine). Alternative crosslinkers may be more or less reactive in polymerization than bis. Therefore, some adjustment in the concentration of initiators may be necessary to achieve optimal polymerization. For a discussion of alternative crosslinkers, see Gelfi and Righetti (1981b).

Gel Additives

The most common gel additives include SDS (sodium dodecyl sulfate), Triton* X-100 detergent, and chaotropic agents such as urea and formamide. Detergents can be added to most common buffer systems without significantly affecting polymerization. Agents such as urea and formamide, however, cause the formation of smaller pore-size gels than would be formed in their absence (urea is often a component of gel systems used to separate small proteins and peptides). This may be due to the disruption of hydrogen bonds between monomer molecules during polymerization. Smaller pore size may also be achieved at higher polymerization temperatures, an effect also attributed to hydrogen bond disruption. Contaminants of gel additives can affect polymerization. Nonionic additives such as urea, formamide, and Triton X-100 can be deionized with a mixed-bed ion exchange resin. Use 10 gm Bio-Rad Ag 5O1 X-8 resin per 100 ml additive solution and let sit overnight. However, removal of nonionic contaminants from nonionic reagents is not practical. Therefore, all additives should be quality-assured for electrophoresis.

Time

Although visible gelation occurs in 15–20 min for chemical polymerization and 30–60 min for photochemical polymerization, polymerization continues much longer (see Figure 1). Ammonium persulfate/TEMED-initiated reactions should be allowed to proceed for 2 hr to ensure maximum reproducibility in gel pore size. Photochemical polymerization (riboflavin-based initiator system) usually proceeds more slowly than chemical polymerization, and is also dependent on light intensity (Shi and Jackowski 1998). However, riboflavin is usually used for polymerization of electrofocusing gels in which separation is based on charge, and for which gel porosity is of secondary importance. Thus these gels can be used shortly after visible gelation without being affected by slight variations in porosity.

Monomer Concentration

The practical range for monomer concentration is between 3%T and 30%T, where %T refers to % (w/v) of total monomer (acrylamide + bis) in solution. A higher concentration of monomer results in faster polymerization. Therefore, changing from 5% gels to 30% gels will probably allow a reduction of 20–50% in the concentration of initiators.

Polymerization Protocols

There are 2 major initiator formulations for acrylamide polymerization. The first, for chemical polymerization, is used for SDS-PAGE and DNA sequencing. Chemical polymerization employs ammonium persulfate and TEMED as initiators. The second, for photochemical polymerization, is used primarily for horizontal electrofocusing gels. Photochemical polymerization calls for riboflavin as well as ammonium persulfate and TEMED. Riboflavin phosphate can be substituted for riboflavin. Riboflavin phosphate is often preferred for its greater solubility.

Preparation for Polymerization

1. Prepare 10% ammonium persulfate shortly prior to use (prepare fresh daily). TEMED is used undiluted. Prepare 0.1% riboflavin (or riboflavin phosphate, which is more soluble) if photopolymerization will be performed.
2. Combine buffer stock solution, monomer stock solution, and water in the appropriate proportions in an Erlenmeyer flask. Since stock solutions are usually stored at 4°C, the gel solution should be allowed to warm to room temperature before degassing.
3. Prepare the gel casting mold, i.e., plates, spacers, and clamps for gel casting. Be sure they are neither hot nor cold.
4. Once the gel solution is prepared and brought to room temperature (23–25°C), degas the solution under a vacuum of 125 torr or better for 15 min at room temperature (for systems in which constant agitation is used during degassing, 10 min is sufficient). Longer periods of degassing are generally not deleterious, although long degassing will result in somewhat faster polymerization.

Chemical Polymerization in Discontinuous Systems — Lower (Resolving) Gel

In a discontinuous system, such as that of Laemmli, the resolving gel is polymerized first. Then, the stacking gel is cast on top of the resolving gel. Use the following protocol to prepare resolving gels for all discontinuous systems (see the next section for preparation of stacking gels).

	Initiator volume per 10 ml gel solution	Initiator final concentration
Ammonium persulfate (10% w/v)	50 µl	0.05%
TEMED (undiluted)	5 µl	0.05%

Swirl the solution gently but thoroughly. Holding the flask by the neck with one hand, swirl it 8 to 10 cycles. This mixes the initiators completely without introducing too much oxygen. Swirling too little can result in uneven polymerization.

Cast the gel by introducing the monomer solution into the gel mold in a steady stream to minimize the introduction of oxygen. Overlay the monomer solution using water, isoamyl alcohol, or water-saturated isobutyl alcohol to exclude oxygen from the surface.

Allow polymerization to occur at room temperature at least 90 min prior to use (see Figure 1).

Chemical Polymerization in Continuous Systems and Stacking Gels

Continuous systems consist of a single gel. Continuous systems are used for some types of protein electrophoresis, and for DNA sequencing. Stacking gels are part of discontinuous systems. These gels have in common contact with the well-forming comb and greater exposure to molecular oxygen at the surface. Use the following levels of initiators for continuous systems and stacking gels.

	Initiator volume per 10 ml gel solution	Initiator final concentration
Ammonium persulfate (10% w/v)	50 µl	0.05%
TEMED (undiluted)	10 µl	0.1%

Swirl the solution gently but thoroughly.

Cast the gel and insert the well-forming comb without trapping air under the teeth.

Allow polymerization to occur at room temperature at least 90 min prior to use (see Figure 1).

Photochemical Polymerization

This protocol is recommended for isoelectric focusing (IEF) gels. Since molecules remain in the IEF gel during electrophoresis, excess ions from initiators can cause distortion of bands. Photochemical initiation is recommended for IEF gels because it is effective at low initiator concentrations.

1. When the gel solution is prepared and brought to room temperature (23–25°C), degas the solution under a vacuum of 125 torr or better for 2 min at room temperature (for systems in which constant agitation is used for degassing, 1 min is sufficient).
2. Add initiators as follows:

	Initiator volume per 10 ml gel solution	Initiator final concentration
Riboflavin (0.1% w/v)	50 µl	0.0005% (5 µg/ml)
Ammonium persulfae (10% w/v)	15 µl	0.015%
TEMED (undiluted)	15 µl	0.05%

3. Swirl the solution gently but thoroughly.
4. Cast the gel and allow polymerization to occur for at least 2 hr for isoelectric focusing gels. If separation is to be based on size, allow photochemically initiated gels, to polymerize for 8 hr under light from a nearby fluorescent lamp, (Righetti et al. 1981).

Polymerization Analysis

There are several ways to assess the extent and reproducibility of polymerization. One of the easiest methods is to routinely monitor the time required for visible gelation. There are several factors which affect the polymerization rate. A significant change in the time required for visible gelation indicates that one of the parameters has changed.

The polymerized gel should be inspected for evidence of inhibition or nonuniform polymerization. A swirled or "schlieren" pattern, for example, indicates that polymerization was too fast or that the polymerization initiators were not mixed thoroughly with the monomer solution prior to casting the gel.

Polymerization Profile

As acrylamide polymerizes, UV-absorbing double bonds are eliminated. The progress of a reaction can therefore be followed by monitoring absorbance at 260 nm. As the reaction proceeds, the UV absorbance drops. Absorbance increases with the amount of unreacted monomer.

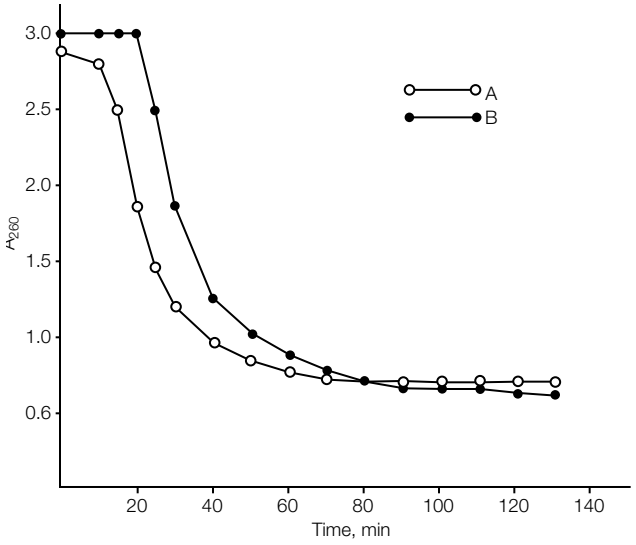


Fig. 1. Polymerization profile for 12% monomer (12%T, 2.6%C*) containing 0.375 M Tris-HCl, pH 8.8 (Laemmli), polymerized in a quartz cuvette at room temperature with ammonium persulfate and TEMED at final concentrations of 0.05% each. Polymerization was monitored at 260 nm. A, "enzyme grade" acrylamide. B, Bio-Rad's electrophoresis-purity acrylamide, control # 25281.

Figure 1 shows a UV profile of chemical polymerization for 2 samples of acrylamide polymerized under identical conditions in a quartz cuvette. Sample A was an "enzyme grade" acrylamide with a conductivity (50% w/w) of 3.75 µS. Sample B was Bio-Rad's electrophoresis-purity acrylamide with a conductivity of 0.56 µS. As the figure shows, polymerization is largely complete after about 90 min, even though the reaction proceeds to a small extend beyond that time. While sample A began to polymerize faster, sample B polymerized more completely, as indicated by the lower final UV absorbance.

Contaminants in acrylamide may be accelerators or inhibitors of polymerization. Therefore, initiation of polymerization, as indicated by reduced absorbance, may be faster with crude acrylamide than with highly refined acrylamide. However, the most important consideration is the completeness of polymerization. Polymerization of highly refined acrylamide may be initiated more slowly, but conversion of monomer to polymer, as indicated by the low final absorbance, is more complete. Therefore, less residual monomer remains. Complete polymerization is critical for reproducibility in gel porosity.

Gel Exclusion Limit Determination

Estimation of protein molecular weight by SDS-PAGE is a widely employed procedure. The relative mobility of a protein in an SDS-PAGE gel is related to its molecular weight. A standard curve is constructed with proteins of known molecular weight by plotting the logarithms of their molecular weights versus the relative mobilities of the proteins. The relative mobility of a protein of unknown molecular weight is then fitted to the curve to determine its molecular weight.

A standard curve can be extrapolated to give the y-intercept, which represents the molecular weight exclusion limit of that particular gel. That is, proteins with a molecular weight greater than the y-intercept value will show zero mobility and will be excluded from the gel matrix.

Poorly polymerized gels have greater porosity due to incomplete chain elongation and crosslinking. As a result, the exclusion limit will be greater than for a well-polymerized gel of the same percent acrylamide. Furthermore, when polymerization is incomplete, exclusion limits are irreproducible. Use of highly purified gel-forming reagents and proper polymerization technique will result in the lowest and most reproducible exclusion limits for a given percent total monomer.

Figure 2 shows a typical curve obtained by plotting log molecular weight versus relative mobility following SDS-PAGE for a group of standard proteins. The antilog of the y-intercept value of this plot is 115,000 as determined by linear regression analysis. The approximate molecular weight exclusion limit of the gel is thus 115,000. The y-intercept value should be considered approximate because it depends upon the relative mobility of the proteins used as standards.

Although the y-intercept value will be different for every gel acrylamide percentage, and slightly different for every set of standards, the value should be highly reproducible from gel to gel if the same acrylamide percentage and standards are used. Thus, monitoring the y-intercept of the log molecular weight vs. relative mobility plot is an excellent assessment of reproducibility in polymerization technique.

* %C = (grams crosslinker x 100)/(grams monomer + grams crosslinker)

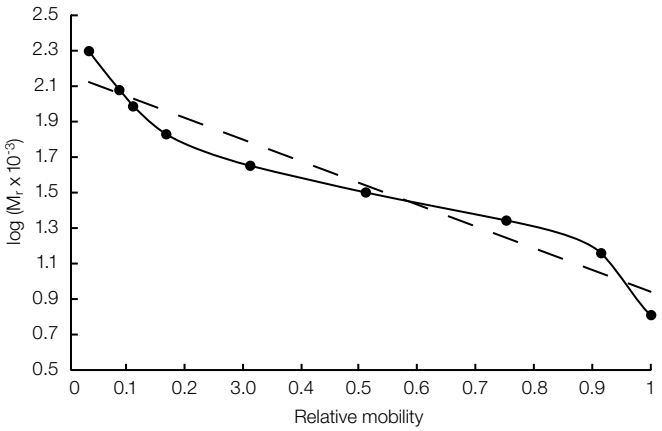


Fig. 2. A representative calibration curve for molecular mass (M_r) estimation. In the run that is plotted here (solid line), Bio-Rad SDS-PAGE standards with M_r of 200, 116.2, 97.4, 66.2, 45, 31, 21.5, 14.4, and 6.5 kD (closed circles, left to right) were separated on a 15%T SDS-PAGE gel. The plot shows the inherent nonlinearity of such curves. The straight-line segment in the middle of the plot is the most accurate range for M_r estimation. Larger polypeptides experience greater sieving than do those in the middle range, so the corresponding upper part of the curve has a different slope than in the middle. The curve in the smaller polypeptide range also deviates from a straight line because of less sieving. Because the scale is logarithmic, an estimate of M_r from a "best fit" straight line (dashed) is acceptable for many purposes.

Handling of Acrylamide

Use good laboratory practices, work in a well ventilated area and wear proper personnel proctective equipment. Refer to the MSDS for further information.

Reagent Storage and Shelf Life

Acrylamide and bis-acrylamide — Electrophoresis-purity acrylamide and bis can be stored dry at room temperature (23–25°C) for at least 1 year.

Ammonium persulfate and potassium persulfate — These initiators can be stored tightly sealed at room temperature for at least 1 year. Solutions should be made fresh daily, since persulfate in solution decomposes rapidly. Persulfate is a strong oxidizing agent. Disposal should be in accordance with local regulations.

TEMED — This initiator can be stored tightly closed either at 4°C or at room temperature for at least 6 months.

After 10 to 12 months, a significant reduction in reactivity requires an increase in the concentration required for proper polymerization. This loss of reactivity is probably due, at least in part, to the gradual accumulation of water.

Riboflavin and riboflavin-5'-phosphate — These photoinitiators can be stored dry at room temperature for at least 1 year. In aqueous solution, they are stable for at least 1 month if kept in the dark at 4°C. Riboflavin phosphate is usually preferred because of its greater solubility.

Polymerization Artifacts and Troubleshooting

Complete and reproducible polymerization is dependent on proper technique and pure gel-forming reagents. The table below lists commonly observed polymerization artifacts and problems, along with their probable cause and solution.

Problem	Cause	Remedy
SDS gel doesn't polymerize	• Too little or too much APS or TEMED	• Requires 0.05% APS and 0.05% TEMED
	• Failure to degas	• Degas 10–15 min
	• Temperature too low	• Cast at room temperature, warming glass plates if necessary
	• Poor-quality acrylamide or bis	• Use electrophoresis-purity reagents
	• APS not freshly made	• Make solution fresh daily
Swirls in gel	• Excessive catalysis — gel polymerized in <10 min	• Reduce APS and TEMED by 25% each
	• Gel inhibition — polymerization time >1 hr	• Increase APS and TEMED by 50%; degas
IEF gel doesn't polymerize	• Basic gradient; gel polymerization problem	• Requires 0.015% APS, 1 µl/ml TEMED and 0.0005% riboflavin
	• Gel has no structure	• Degas 1–2 min
	• Riboflavin-catalyzed; gel was degassed too long	• O_2 required for this reaction to initiate — don't degas
	• APS alone doesn't polymerize	• Requires 0.015% APS, 1 µl/ml TEMED
	• Didn't degas for chemical polymerization. Temperature too low.	• Degas 10–15 min at room temperature
Long polymerization time, incomplete catalysis	• Poor-quality acryl-amide or bis	• Use electrophoresis-purity acrylamide and bis
	• APS not freshly made	• Make fresh APS
	• Failure to degas	• Degas 10–15 min
	• Too little APS or TEMED	• Increase both to 0.05%
	• Temperature too low	• Cast at room temperature
	• Poor-quality acryl-amide or bis	• Use electrophoresis-purity reagents
	• APS not freshly made	• Make APS fresh daily
	• TEMED old	• Use new TEMED

Problem	Cause	Remedy
Gel feels soft	• Low % T	• No remedy
	• Poor-quality acryl-amide or bis	• Use electrophoresis-purity reagents
	• Too little crosslinker	• Make sure of proper %C
Gel turns white	• bis concentration too high	• Recheck solutions or weights
Gel brittle	• Crosslinker too high	• Recheck %C
Diffuse or broad bands	• Poor-quality acrylamide or bis; incomplete catalysis	• Use electrophoresis-purity reagents
	• Sample not equilibrated	• Equilibrate sample to running conditions
	• Excessive TEMED or APS	• Reduce initiator concentrations by 25%
	• SDS or sample buffer too old	• Prepare fresh solutions
	• Gel temperature high	• Cool during run or run more slowly
Inconsistent relative mobilities	• Incomplete catalysis; excessive TEMED or APS	• TEMED and APS should be 0.05%
	• Did not degas	• Degas 10–15 min
High silver-staining background	• Acrylic acid contamination in acrylamide and bis	• Use electrophoresis-purity reagents
Severe cathodic drift in IEF, molecules diffuse or don't reach proper pI	• Acrylic acid contamination in acrylamide and bis	• Use electrophoresis-purity reagents
	• Gel not aged long enough after photopolymerization	• Allow polymerization for 8 hr before using the gel
	• Poor-quality urea	• Deionize urea; use electrophoresis-purity reagents

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