

# Development of a Clear, Photopolymerizable Acrylamide Gel and Its Use In Immobilizing and Staining Nucleic Acids.

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## Abstract

*A clear, photopolymerizable gel of a polyacrylamide has been developed. The components will form a gel in presence of high concentrations of cesium chloride. DNA immobilized in such a gel can be stained and the blank gel subsequently destained. The application of this technique to isopycnic density gradient centrifugation of cesium chloride solutions was discussed.*

## Introduction

Polymerization of acrylamide and N,N'-methylene-bis-acrylamide has been widely used in the preparative, routine and analytical versions of disc electrophoresis (1). Additionally, photopolymerization of these compounds have been used in conjunction with sucrose density gradient centrifugation for immobilization of components separated by centrifugation (2, 3, 4). This report extends the immobilization technique to dense solutions of cesium chloride and details the development of a clear photopolymerizable gel. The techniques of staining and destaining the immobilized gel are reported also.

## Methods and Materials

### Preparation of Cesium Chloride-Containing Gels

Cesium Chloride (SC11352) was purchased from the Sargent-Welch Company. Cesium chloride was dissolved in water to give a solution with a density of around 1.875 gm/ml. Such a solution requires about 1.200 gms CsCl/ml solution at 20°C. Three parts of the cesium chloride solution were mixed with one part of polymerizable solution.

Polymerizable solution is made of three parts, Solutions B and C and riboflavin. Solution B consists of 11.4 gm Tris buffer, 1.2 ml N,N,N',N'-tetramethylethylenediamine (TEMED, practical grade, Matheson Coleman and Bell 8563) and distilled water to a final volume of 33 ml. The pH is adjusted to 6.9 with 85 percent phosphoric acid. Solution C is 24 gm of acrylamide, 0.735 gm N,N'-methylene-bisacrylamide (Bis, Eastman 8883) and distilled water to give a final volume of 50 ml. Approximately 0.5 mg riboflavin/5 ml aliquot is dissolved in the polymerizable solution. Solutions B and C are stable and may be stored at room temperature. After addition of riboflavin the polymerizable solution must be protected from light.

Layers of gel were successively polymerized in a cellulose nitrate centrifuge tube. Alternate layers contained DNA. Each layer was polymerized from 0.4 ml of solution (0.1 ml of Cesium chloride solution, 0.3 ml of polymerizable solution) with a thin (2 mm) overlay of water. Those layers which contained DNA were made by diluting stock DNA

solution with cesium chloride-polymerizable solution. Polymerization was completed for each layer in 10 to 20 minutes at a distance of 4 inches from a 15 watt fluorescent bulb.

### Staining and Destaining

After polymerization the gels were removed from the cellulose nitrate tubes by shaking or rimming with a microspatula and were stained by one of a variety of procedures. Three procedures gave the best results and they are reported here. A methyl green procedure (5, 6) gives a green stain, pyronin B (5) gives a lavender stain and methyl green and pyronin B together (7) give a blue-green stain. The gels were immersed in the staining solution for 1 hour or more. The excess stain was removed by repeated washing in 0.2N acetate buffer of an appropriate pH (4.0 for methyl green, 4.5 for pyronin B and 4.25 for methyl green-pyronin B). Electrophoretic destaining may be done in the same buffers. A suitable destaining apparatus may be constructed from lucite (8). The washing procedure takes about 2 days with occasional changes of buffer. The electrophoretic procedure requires about 24 hours at 3-4 ma of current per tube.

### DNA Samples

Calf thymus DNA (Sodium Salt, Type I, lot 115B-1690) was purchased from Sigma Chemical Company, St. Louis, Missouri. A stock solution of DNA was prepared by dissolving DNA in dilute NaCl-citrate (0.015M NaCl, 0.0015M sodium citrate, pH 7).

### Results

The results show that within the range of approximately 100 to 250  $\mu\text{g}$  calf thymus DNA/ml good contrast bands were obtained. At 500  $\mu\text{g}$ /ml the DNA partially inhibited the polymerization process. Consequently, the 500  $\mu\text{g}$ /ml band was small due to loss of material.

### Discussion

The photopolymerizable gel of disc electrophoresis (1) and sucrose density gradient techniques (2, 3, 4) is translucent. The transparent gel reported here is the result of lowering the Bis/acrylamide ratio. This means that the transparent, photopolymerizable gel is not as highly cross-linked as the translucent one.

The results reported here show that DNA can be immobilized, stained and destained in a gel containing cesium chloride at concentrations comparable to those used in isopycnic density gradient centrifugation. Thus, cesium chloride does not interfere with the polymerization process.

The methyl green stain has been used successfully for staining native DNA in acrylamide gels (5); since these gels are much thicker than those of disc electrophoresis the primary exposure time to the stain was 1½ more hours rather than 1 hour as reported by Boyd and

Mitchell. The pyronin B method has been used for denatured DNA (5). Presumably, our DNA samples were not denatured and the pyronin B method gave the least satisfactory results. The methyl green-pyronin B method gave the best contrast but the methyl green alone could be completely removed from non-DNA containing areas of the gel.

With the techniques developed here, it should be possible to carry out isopycnic cesium chloride density gradient centrifugation with DNA and stain the samples after polymerization. With disc electrophoresis many staining procedures have been quantitated with a microdensitometer scanning of the gel (1). This instrument costs \$3900. Recently, an inexpensive high-resolution densitometer for disc electrophoresis has been developed (9). The total cost for materials for this instrument are reported not to exceed \$300. Thus, a quantitative and analytical data-acquisition system for density gradient centrifugation should be possible without the use of an analytical ultracentrifuge (Model E, Spinco Division, Beckman Instruments, Inc., Palo Alto, California 94304) or drop collecting techniques. The requirements for such a system are a preparative ultracentrifuge, the immobilization and staining procedure reported here, and a microdensitometer for gel scanning.

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