# A Convenient Procedure for Transfer Blotting of Coomassie Blue Stained Proteins from PAGE Gels to Transparencies


## ABSTRACT

*Proteins stained with Coomassie Brilliant Blue* ***1*** *were transferred effectively by blotting from polyacrylamide gel electrophoresis (PAGE) gels to transparencies of the type used in plain-paper copiers. The details of the original electropherogram were retained on transfer and did not fade over a period of three years. Both the protein and the associated dye transfer; however, protein does not transfer in the absence of dye. Protein patterns present in several types of gels—sodium dodecyl sulfate (SDS)-PAGE, non-denaturing PAGE, isoelectric focusing PAGE and SDS-agarose—all transferred successfully after staining with dye* ***1****. Proteins visualized with other organic dyes such as Fast Green FCF* ***2****, Uniblue A* ***3*** *and Procion Blue MX-R* ***4*** *also transferred, but proteins stained with Stains-all* ***5*** *or silver did not. This transfer provides a simple, economical way to preserve data from slab gel electrophoresis and a convenient method to display data using an overhead projector. The blotted transparencies are also excellent substrates for use with gel scanning densitometers.*


## INTRODUCTION

Polyacrylamide gel electrophoresis (PAGE) is widely used for analysis of proteins and nucleic acids (2,9,13,15). After electrophoresis, proteins are often visualized by staining the gel with Coomassie Brilliant Blue **1** (Figure 1). This dye has a high extinction coefficient in the visible spectrum upon complexation (16) and a high affinity for proteins that does not depend strongly on the specific structures of the proteins (3). Other dyes (Figure 1), such as Fast Green FCF **2** (1), Uniblue A **3** (5), Procion Blue MX-R **4** (6) and Stains-all **5** (8), are also used occasionally, although these dyes are about ten times less sensitive than dye **1** for staining proteins.

For storage, PAGE gels are often treated in one of two ways. They can be placed in sealed plastic bags containing 7% aqueous acetic acid (9). More commonly, these gels are photographed and dried. In lieu of storage, when further manipulation of the proteins in the gels is to be done, the pattern is transferred to blotting membranes.

Transfer blotting of proteins from PAGE gels to other films has been examined previously (7). Electroblotting, diffusion and convection blotting are three common techniques for transfer of proteins (7). Nitrocellulose membranes, diazotized papers, nylon-based membranes and polyvinylidene fluoride (PVDF) membranes are commonly used matrices for transfer blotting (12).

Here we describe a convenient, inexpensive method for long-term non-photographic storage of the information present in electrophoresis gels stained with dyes based on blotting the stained gels onto commercial transparencies of the type used in preparing viewgraphs for overhead projection. The dye-stained transparencies faithfully replicate the information present in the originally stained gel. They are convenient to store. They are also excellent substrates for use with gel scanners, since they are flat, dimensionally stable, and have no color themselves.

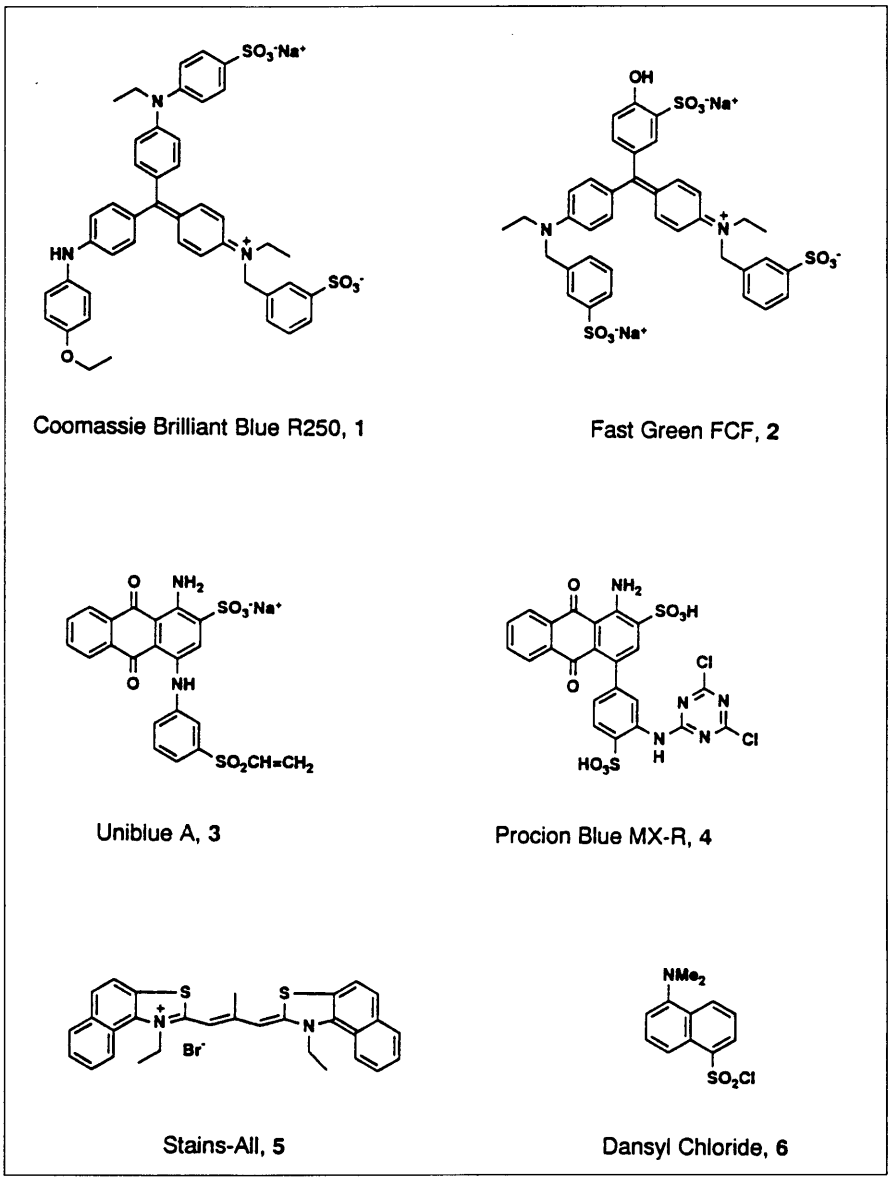


**Figure 1. Chemical structures of organic dyes 1–6 used.**

Table 1. Preparation of Solutions of Dyes 1–5 Used for Staining Protein Gels

| Dye | Concentration (wt/vol) | Staining Solution (vol/vol/vol) |
|---|---|---|
| 1 | 0.1% | $CH_3OH/H_2O/HOAc$* (4:5:1) |
| 2 | 0.1% | $C_2H_5OH/H_2O/HOAc$ (3:6:1) |
| 3 | 0.6% | $CH_3OH/H_2O/HOAc$ (5:4:1) |
| 4 | 0.05% | $CH_3OH/H_2O/HOAc$ (4:5:1) |
| 5 | 0.01% | $HCONH_2$/iPrOH/ Tris-HCl (15 mM, pH 8.8) (2:5:13) |

*Ac = acetate

## MATERIALS AND METHODS

Chemicals used for electrophoresis were obtained from Bio-Rad (Hercules, CA). Organic dyes Coomassie Brilliant Blue **1**, Fast Green FCF **2**, Uniblue A **3** and dansyl chloride **6** were purchased from Sigma Chemical (St. Louis, MO). The Procion Blue MX-R **4** and Stains-all **5** were obtained from Fluka (Ronkonkoma, NY) and Aldrich (Milwaukee, WI), respectively. The transparency sheets were the type intended for use in plain-paper copiers. Eight commercial transparencies were tested for transfer blotting: Arkwright PPC-IX (Arkwright, Fiskeville, RI), ACP (American Coated Products, Clinton, CT), Labelon (Canandaigua, NY), 3M PP2500 (3M Visual Systems Division, Austin, TX), Pitney-Bowes (Stamford, CT), Polaroid 731 (Polaroid, Cambridge, MA), Savin (Brighton, MA), Universal (United Stationers Supply, Des Plains, IL). Membranes used for nucleic acid and protein blot were DEAE-cellulose (Schleicher & Schuell, Keene, NH), nitrocellulose and Zeta-Probe® (Bio-Rad).

### Preparation of Bovine Serum Albumin (BSA) Conjugates of 3, 4 and 6

To a solution of BSA (100 mg, 1.5 mmol) in 0.1 M sodium borate buffer (9 ml, pH 9.0) were added **3**, **4** or **6** (15 mmol) as the solid at room temperature. The reaction was stirred at room temperature for 2 h and then subjected to dialysis against water (2 × 4 liters) for 48 h at 4°C. The dialyzed protein solution was lyophilized. BSA-**3** and BSA-**4** conjugates were isolated as blue powders and the BSA-**6** conjugate as a yellow powder. All protein conjugates were stored at 4°C.

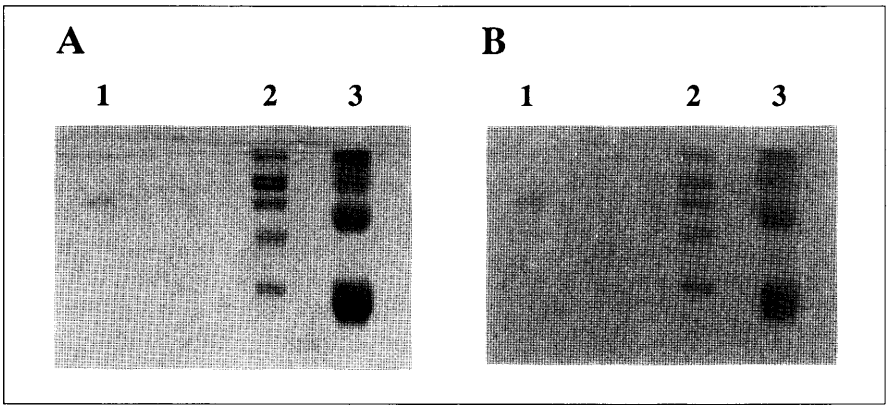


**Figure 2. Comparison of PAGE gels with the transfer blots onto transparencies.** (A) An SDS-PAGE gel (12% T [acrylamide], 0.3% C [cross-linker]) of mixtures of proteins stained with Coomassie Blue **1**. Lane 1, serum albumin from bovine; lane 2, protein mol wt standards: (from bottom to top) carbonic anhydrase (30000; from bovine erythrocyte), albumin (45000; from egg), serum albumin (66000; from bovine), phosphorylase b (97400; from rabbit muscle), β-galactosidase (116000; from *E. coli*), myosin (205000; from rabbit muscle); lane 3, oligomers of bovine carbonic anhydrase cross-linked by dimethyl subserimidate. (B) Transfer blot of A onto transparency.

### Gel Electrophoresis and Transfer Blotting

Gel electrophoresis was carried out following the Laemmli procedure for sodium dodecyl sulfate (SDS)-PAGE (10) and the Margolis procedure for non-denaturing PAGE (11). The procedure available in Hames and Rickwoods was used for isoelectric focusing (IEF)-PAGE (2). SDS-agarose electrophoresis was carried out following the manufacturer's procedure (ProSieve® gels; FMC BioProducts, Rockland, ME). Electrophoresis used the Bio-Rad mini PROTEAN® II system for denaturing and non-denaturing PAGE and the Multiphor apparatus (Pharmacia Biotech, Piscataway, NJ) for IEF-PAGE. After the electrophoresis, the gels were stained with dyes **1–5** (Table 1).

The gels were destained using the same solutions without dye. The destained gels were removed from the destaining solution and immediately placed between two sheets of transparency. Both sides of the transparency were tested for blotting. Since this diffusion blotting from the PAGE gel can take place from either side of the gel, this procedure gives two mirror images but otherwise indistinguishable replicas of the original PAGE gel. The transfer was allowed to proceed at room temperature for 2 h or 30 min at 40°C, the sandwich was disassembled and the transparencies were air-dried and stored.

## RESULTS AND DISCUSSION

### Procedure Using Coomassie Dye 1

When a polyacrylamide gel containing proteins was stained with Coomassie Brilliant Blue **1** and the gel was pressed against the transparency for 2 h at room temperature or 30 min at 40°C, the blue bands characteristic of the protein-dye complexes transferred to the transparency. Figure 2 shows representative results obtained with mixtures of proteins that were separated by SDS-PAGE (10). At the usual level of resolution, the transfer faithfully reproduced the original. The transfer of color from the PAGE gel to the transparency was accompanied by the transfer of protein: when the part of the surface layer that contained the blue band was scraped as a powder from the surface of the transparency and subjected to protein analysis (i.e., hydrolysis in 6 *N* HCl and treatment with ninhydrin), amino acids were detected. The dye alone did not give a positive ninhydrin test (14). The dye **1** appeared to decompose under the conditions used to hydrolyze proteins (6 *N* HCl, 110°C, 24 h): it became colorless and showed a negative ninhydrin test. The transferred Arkwright transparency showed about ten times stronger ninhydrin test than the transparency alone (with protein carbonic anhydrase used as the example) under the same experimental condition (i.e., same dimensions of films and volumes of ninhydrin reagent). The Mylar® film and the transparency from Pitney Bowes showed a negative ninhydrin test. When we resubjected the samples obtained by scraping blue bands from the transparency to electrophoresis, the corresponding protein bands were observed, although the bands were blurred (we used a silver stain in these experiments).

We were not able to electrophoretically blot dye-protein complexes from PAGE gels to transparencies. We presume that the transparencies are poreless and not conductive in an electric field.

When the transparency was pretreated with reagents, such as the gel-destaining solution, organic solvents (acetone, dimethylformamide and dimethyl sulfoxide) and protein-denaturing agents (6 M guanidine hydrochloride, 1% SDS), transfer of the protein-dye complexes to the transparency still occurred. When transparencies had been pretreated with hot acetic acid, which separates the thin coating films on which the image forms from the structural body of the transparency (the Mylar backing), transfer did *not* occur.

When the electrophoresis was conducted in the non-denaturing buffer, blotting transferred the protein-dye complexes to the transparency. The proteins on an IEF-PAGE gel stained with **1** readily transferred to the transparency. When protein electrophoresis was carried out using an agarose matrix rather than polyacrylamide, the blotting worked as well as it did with SDS-PAGE gel. These results indicate that neither the gel matrix (polyacrylamide or agarose) nor the type of electrophoresis (denaturing, non-denaturing or isoelectric focusing) influence the protein blotting from the gel to the transparency.

### Transparencies, Films and Membranes

We surveyed a number of commercial transparencies used for plain-paper copiers, organic films commonly used for the backing supports of transparencies and filtration membranes, and membranes used for nucleic acids and protein blottings to determine the generality of the transfer blotting (see Materials and Methods). We found that the blotting membranes nitrocellulose, the Zeta-Probe and DEAE-cellulose worked as expected, but that only two (the Arkwright PPC-IX and Polaroid 731) out of eight transparencies tested transferred the dye-protein complexes from the gel to the transparency. Both sides of the transparency accepted proteins. Neither the uncoated Mylar film nor the cellulose film worked.

### Other Dyes

We surveyed four other dyes **2**, **3**, **4** and **5**, which are used less frequently than **1** to stain gels, for utility in this blotting technique. We found that all protein patterns formed by staining PAGE gels with dyes **2**, **3** and **4** transferred faithfully to transparencies. Dyes **1–4** have two key features in common: all are anionic and hydrophobic. The proteins stained by the cationic dye **5**, however, did not transfer. These dyes do not react chemically with proteins but only form non-covalent complexes (4). Not surprisingly, gels developed using other types of procedures (e.g., staining with silver or gold) did not transfer during blotting.

### Modification of the Protein

Protein only transferred from gel to transparency when the gel had been

stained with dyes. The association between dye and protein involves some combination of hydrophobic and electrostatic interactions (4). In an effort to increase both effects, we carried out PAGE on the covalently linked BSA conjugates of **3**, **4** and **6** for two reasons. The BSA-**3** and BSA-**4** conjugates have greater capacity for both electrostatic (by the sulfonate groups) and hydrophobic (by the arene groups) interactions and are intensely colored themselves. The BSA-**6** conjugate is more hydrophobic, due to the introduction of a naphthalene group, and is fluorescent. We found that both BSA-**4** and BSA-**5** conjugates were transferred from the gel to the transparency, without additional staining with dyes in the acidic medium. Qualitatively, staining these BSA-**4** and BSA-**5** conjugates with Coomassie dye **1** increased the efficiency of transfer. The BSA-**6** conjugate did not transfer alone, but the complex of the dye **1** and the BSA-**6** conjugate successfully transferred.

These results suggest that anionic sulfonate groups present in dyes associate electrostatically with proteins and that this association facilitates transfer blotting. Hydrophobic interactions appear less important for the transfer of protein-dye complexes to transparencies (4).

## Advantages

We have described a convenient method for long-term, non-photographic storage of the information present in electrophoresis gels, based on blotting protein patterns, after staining with Coomassie Brilliant Blue and other dyes, onto commercial transparencies. These transparencies are readily available and inexpensive. The blotted transparencies retain the information present in the originally stained gel. They are dimensionally stable, mechanically sturdy, flat and convenient to store. This method provides a simple, convenient and inexpensive way to preserve data from protein electrophoresis and an effective method of displaying data using an overhead projector (in particular, the patterns in the transparency preserved their original colors on transfer). These dye-stained transparencies are also excellent substrates for use with gel scanning densitometers.

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**Yen-Ho Chu and George M. Whitesides**
*Department of Chemistry*
*Harvard University*
*12 Oxford Street*
*Cambridge, MA 02138*