Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

A Comparison of Glycerol and Aqueous Methanol Equilibration for Increased Resolution during Two-Dimensional Electrophoresis

Thomas Marshall[a]
[a] THE BIOLOGY DEPARTMENT, THE UNIVERSITY OF ULSTER AT COLERAINE, COLERAINE, NORTHERN IRELAND

To cite this Article Marshall, Thomas(1986) 'A Comparison of Glycerol and Aqueous Methanol Equilibration for Increased Resolution during Two-Dimensional Electrophoresis', Separation Science and Technology, 21: 4, 433 — 437
To link to this Article: DOI: 10.1080/01496398608057173
URL: http://dx.doi.org/10.1080/01496398608057173

SEPARATION SCIENCE AND TECHNOLOGY, 21(4), pp. 433-437, 1986

NOTE

A Comparison of Glycerol and Aqueous Methanol Equilibration for Increased Resolution during Two-Dimensional Electrophoresis

THOMAS MARSHALL

THE BIOLOGY DEPARTMENT
THE UNIVERSITY OF ULSTER AT COLERAINE
COLERAINE BT52 1SA, NORTHERN IRELAND

INTRODUCTION

The O'Farrell method of high resolution two-dimensional electrophoresis (*1*) is widely used for analysis of complex protein mixtures (*2, 3*). Among the described modifications to this technique are changes in the sodium dodecyl sulfate (SDS) equilibration step associated with the transfer of first dimension isoelectric focusing (IEF) gels to second dimension polyacrylamide electrophoresis gels (*4-7*). Wright (*8*) has recently recommended SDS equilibration in aqueous 55% methanol for improved resolution. Since we have encountered problems applying this approach to our particular two-dimensional gel system, a simpler, rapid, and more convenient method based on IEF gel shrinkage in 100% glycerol is now recommended.

MATERIALS AND METHODS

Acrylamide, N,N'-methylenebisacrylamide, 2-mercaptoethanol, glycerol, glycine, and SDS were all of electrophoresis or Analar grade and were purchased from BDH Chemicals, Poole, Dorset, UK. Ampholines were purchased from LKB Bromma, Sweden.

The technique of high resolution two-dimensional electrophoresis was

 0149-6395/86/2104-0433$3.50/0

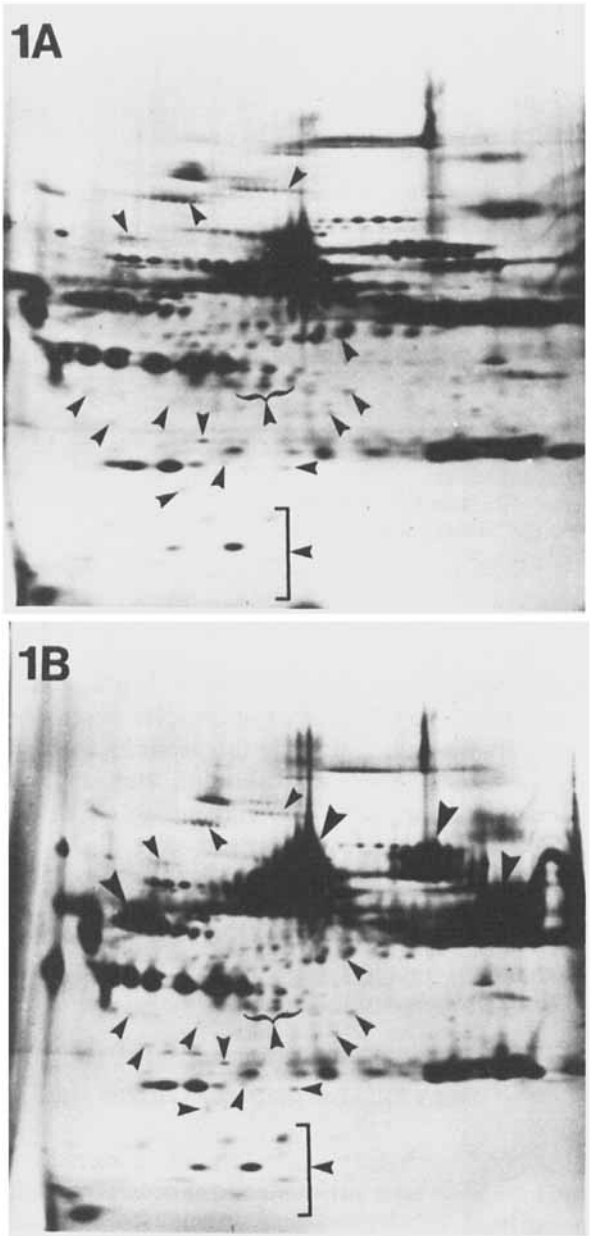
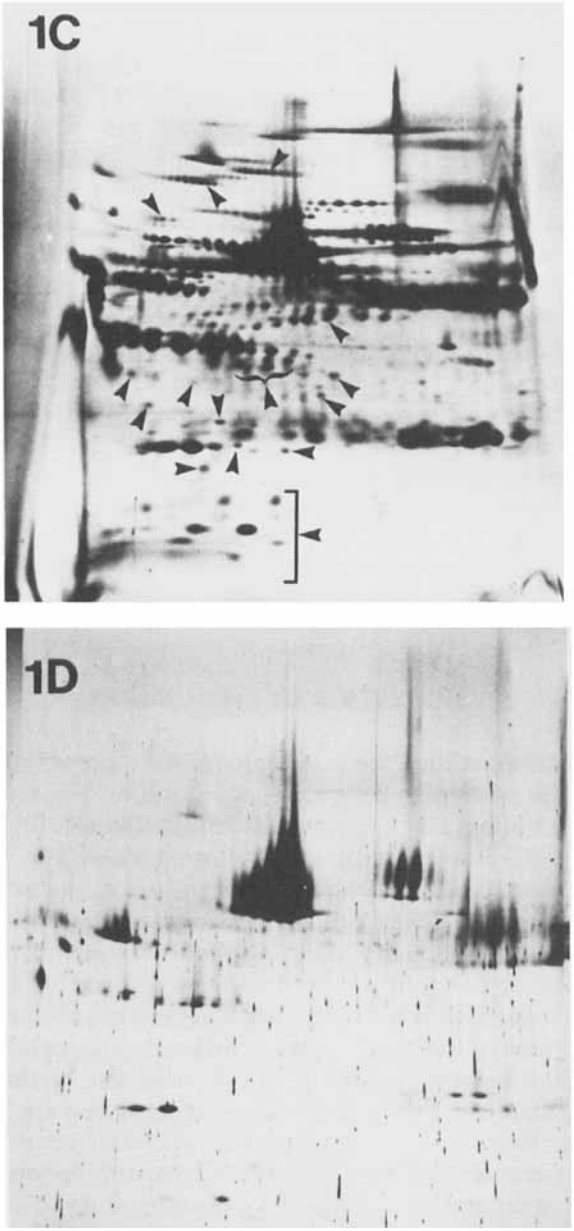


FIG. 1. Silver stained two-dimensional polypeptide patterns of human serum (0.1 μL) following IEF gel equilibration (without 2-mercaptoethanol) in either O'Farrell equilibration buffer (A), aqueous 55% methanol (B), or 100% glycerol (C). Small arrowheads (A–C) indicate representative spots for assessment of resolution. Large arrowheads (B) indicate



protein trailing. The latter was not eliminated by inclusion of 2-mercaptoethanol in the aqueous 55% methanol (D)—this resulted in additional artifacts (vertical streaks) and a general loss of silver stain sensitivity (B, D). In each case the anode of the IEF gel was placed to the left and electrophoresis was performed from top to bottom.

performed as previously described (*6, 9*) with minor modifications. Briefly, IEF was carried out in 4% (w/v) polyacrylamide gel cylinders (65 mm × 3 mm diameter) containing 9 *M* urea, 0.5 *M* Nonidet P-40, and 2% (w/v) Ampholine (pH ranges 3.5–10 and 5–7 (1:4, v/v)). The samples (denatured by heating at 95°C for 5 min in 0.0625 *M* Tris HCl pH 6.8 containing 2% (w/v) SDS and 5% (v/v) 2-mercaptoethanol (*9*)) were applied to the cathode ends of the IEF gels and focusing continued for 17 h at 330 V with a 90-kΩ resistor in series. Duplicate IEF gels were subsequently electrophoresed (SDS-polyacrylamide gel electrophoresis (PAGE)) on 4–20% (w/v) polyacrylamide gel slabs (75 × 75 × 3 mm) following equilibration in either: (i) O'Farrell equilibration buffer for 1 h, 0.0625 *M* Tris HCl pH 6.8 containing 2% (w/v) SDS and 10% (v/v) glycerol (with or without inclusion of 5% (v/v) 2-mercaptoethanol) (*1*); or (ii) Wright equilibration buffer for 1 h, the same buffer as in (i) but containing twice the amount of SDS and diluted with methanol to a final methanol concentration of 55% (v/v) (*8*); or (iii) 100% glycerol for 10 min.

The two-dimensional polypeptide patterns were subsequently visualized by silver staining (*10*).

RESULTS AND DISCUSSION

Figure 1 demonstrates the two-dimensional polypeptide patterns of human serum obtained following equilibration in either O'Farrell equilibration buffer (Fig. 1A), aqueous 55% methanol (Fig. 1B), or 100% glycerol (Fig. 1C). For the purpose of this comparison, 2-mercaptoethanol was omitted from the equilibration solution since its inclusion invariably resulted in electrophoretic artifacts (*11*) and a general impairment of silver stain sensitivity (Fig. 1D). Optimal resolution was obtained following IEF gel equilibration in 100% glycerol (Fig. 1C). Some improvement was also evident after aqueous methanol equilibration, but the enhancement of trailing from the prominent polypeptide constituents proved a major disadvantage (Fig. 1B). In addition, methanol rendered the IEF gels sticky and difficult to manipulate during application to the second dimension. No such problems were encountered with the glycerol although the excess should be removed by draining for approximately 2 min through a tea strainer. Glycerol was also more rapid and effective in achieving IEF gel shrinkage, i.e., a 25% length reduction in 10 min as compared to only 10% in 1 h in aqueous 55% methanol. It seems likely that IEF gel shrinkage effectively sharpens polypeptide bands, thereby

restricting diffusion during protein elution from the first to the second dimension gel. This contrasts with the proposed mechanism of methanol fixation involving *in situ* precipitation of focused proteins (*8*) which probably enhances protein trailing (Fig. 1C) as a result of solubility problems.

REFERENCES

1. P. H. O'Farrell, *J. Biol Chem., 250*, 4007–4021 (1975).
2. M. J. Dunn and A. H. M. Burghes, *Electrophoresis, 4*, 97–116 (1983).
3. M. J. Dunn and A. H. M. Burghes, *Ibid., 4*, 173–189 (1983).
4. G. F.-L. Ames and K. Nikaido, *Biochemistry, 15*, 616–623 (1976).
5. J. I. Garrels, *J. Biol. Chem., 254*, 7961–7977 (1979).
6. T. Marshall, *Electrophoresis, 5*, 245–250 (1984).
7. T. Marshall, K. M. Williams, and O. Vesterberg, *Clin. Chem., 30*, 2008–2013 (1984).
8. W. E. Wright, *Sep. Sci. Technol., 17*, 1689–1696 (1983).
9. A. L. Latner, T. Marshall, and M. Gambie, *Clin. Chim. Acta, 103*, 51–59 (1980).
10. T. Marshall, *Anal. Biochem., 136*, 340–346 (1984).
11. T. Marshall and K. M. Williams, *Ibid., 139*, 502–505 (1984).

Received by editor June 19, 1985