The staining of starch gels with Coomassie Brilliant Blue G 250 perchloric acid solution

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Summary. Coomassie Brilliant Blue G250 dissolved in dilute perchloric acid solution was used to stain protein bands in starch gels. The bands were visible within min, no destaining was required and the gels could be stored indefinitely.

The use of the textile dye Coomassie Brilliant Blue G250 dissolved in dilute perchloric acid (PCA) to stain proteins in polyacrylamide gels was reported recently[1]. The method has the advantage of requiring no destaining and of being rapid and simple. With the modifications outlined below it is possible to use Coomassie Blue G250-PCA to stain proteins in starch gels. The protein bands are observable within minutes of introducing the stain and the gels can be treated to allow them to be kept as permanent records.

For the analyses of sheep serum transferrins, starch gels were prepared and run according to Kristjansson's method[2]. Analyses of sheep red cell haemolysates were undertaken in 10% starch gels (w/v) using Hiller starch (Electrostarch Co., Madison, Wisconsin, USA). The gel buffer consisted of 0.045 M tris (Sigma 7–9) and 0.025 M boric acid (pH 8.5) while the electrolyte buffer consisted of 0.1 M tris (Sigma 7–9) and 0.55 M boric acid (pH 8.5). (Full details of these analyses will be published elsewhere [Piper and McFarland, in preparation]). Haemolysates, prepared by mixing washed red cells with distilled water (1:1, v/v), were absorbed into filter paper strips which were then inserted into the starch gel. A potential of 150 V was then applied to the gel for 30 min at room temperature. The paper strips were removed and the electrophoresis continued for 2.5–3 h at 300 V in the cold room (4 °C).

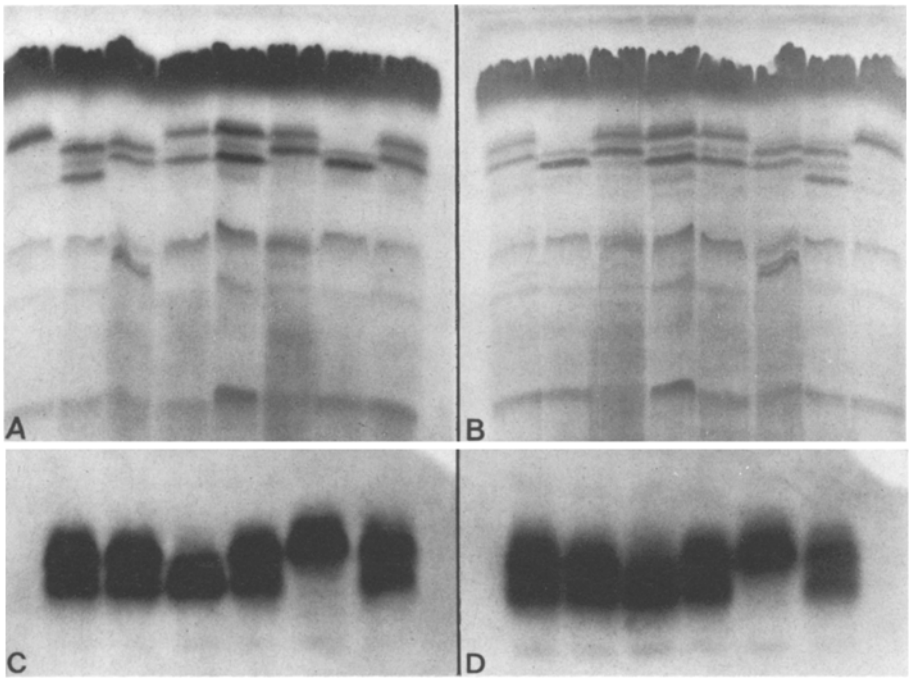
The Coomassie Brilliant Blue G250 (Serva, Heidelberg) PCA solution was prepared as an 0.022% solution (w/v) in 5.8% (w/v) PCA. It was found convenient to make up the dye as an 0.25% (w/v) aqueous stock solution which, after filtering through an 0.45 μm millipore filter, was added to the dilute PCA. Typically, 100 ml of the filtered aqueous dye solution was added to 1000 ml of distilled water and 55.3 ml of 70% PCA (density = 1.72) was then stirred in. This mixture was stable at room temperature indefinitely.

After slicing the starch gel in the usual manner, a slab was immersed in the stain and left for 15–20 min. During this step the gel became very fragile (sloppy) and no attempt was made to handle it. However, stained protein bands were observed to form within 7–15 min. Excess stain was then removed after 20 min by aspiration and distilled water carefully added to rinse the gel. After the aspiration of the water, the rinse was repeated and finally a mixture of methanol-acetic acid-water 5:1:5 (v/v/v) was added in order to fix the gel. The gel was allowed to harden for 1–2 h; during this time some shrinkage occurred, however, it did not affect the resolution of the protein bands (figure). Finally, the methanol-acetic acid-water mixture was removed and the gel stored at 4 °C for as long as required. Starch gels treated in this way were as stable as those stained using routine procedures.

As can be seen in the figure, both serum proteins and haemoglobins are visualized as well by the G250-PCA stain as they are by Nigrosine or Amido Black. However, the principle advantage of using the G250-PCA stain is that proteins can be visualized within min and no destaining of the gels is required.

1 A. H. Reisner, P. Nemes and C. Bucholtz, Analyt. Biochem. *64*, 509 (1975).

2 F. K. Kristjansson, Genetics *48*, 1059 (1963).



Starch gel electrophorograms stained with various dyes. Starch gels were prepared and run as described in the text. The protein solution (neat for sheep serum, diluted 1:1 with distilled water for sheep haemoglobin) was allowed to saturate a 10 × 5 mm strip of Whatman 3 mm paper. The paper was then blotted to remove excess solution and inserted into the gel. *A* and *B* are mirror images so that the stained surfaces seen are contiguous. The anode is at the top of each gel. *A* Sheep serum proteins stained 15 min with G250-PCA solution and then photographed after fixing. *B* Sheep serum proteins stained overnight with Nigrosine before being photographed. *C* Sheep haemoglobins after 10 min staining with G250-PCA. *D* Sheep haemoglobins stained with Amido Black and then destained overnight.