Use of Farmer´s Reducer

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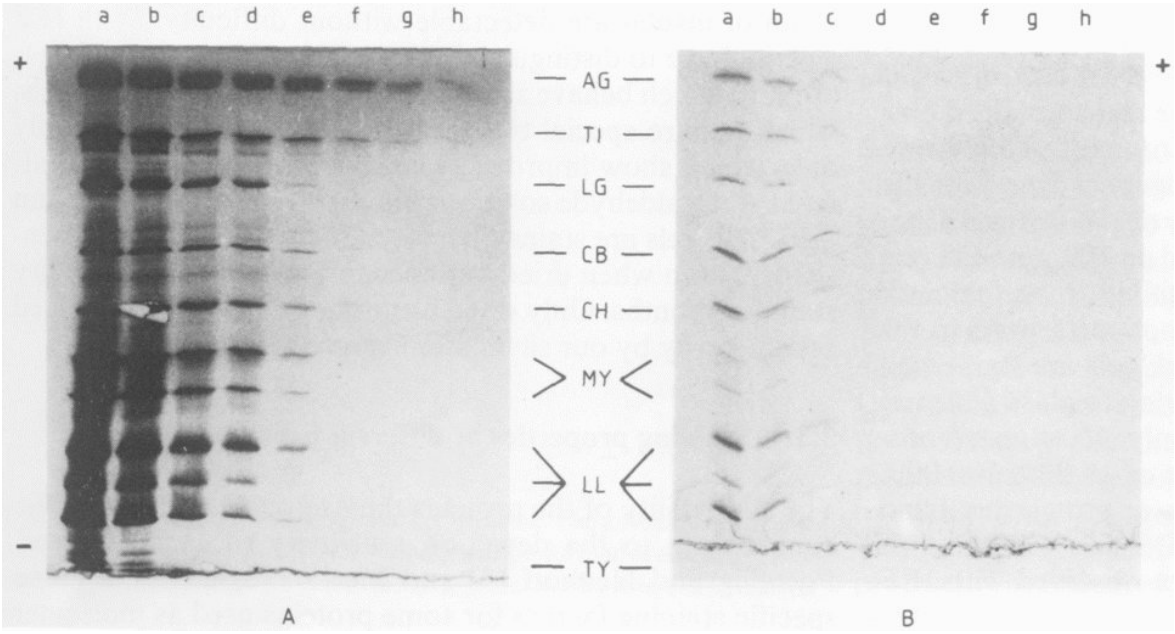
The silver-staining developes to fast and the result is too dark? Then with the help of Farmer´s reducer, a chemical product of the photograph supply, can reduce the colour to a blank gel and the silver-staining can be redone a second time (recycling).



3.2.4 Use of Farmer's reducer and recycling

Brief rinsing after soaking the gel in silver nitrate decreased the formation of the grey coating on the surface but did not prevent it. These light silver deposits can be better removed with a photographic reducer than with clearing solutions commonly used for lighting up photographic films. However, the application of reducers has only been reported in a few instances [2-4, 6, 13]. Oakley [2] mentioned Farmer's reducer without actually using it. Farmer's reducer is a so-called subproportional reducer which reduces mainly the less intense darkening or shadows as well as fogs. Treatment with a reducer solution removes the weak grey film on the gel surface and the slight shading, while at the same time increasing the contrast. More over, the subsequent recycling step is particularly promoted. An enhancer effect can also be seen when the gel is placed in Farmer's reducer for 10 s before the first impregnation with AgN03- In contrast to other reducer systems t 13, 31], Farmer's reducer is applied at a low concentration (0.5 %) for a very short time (10–30 s), followed by quick washing off under running water. This ensures precise control of the reducer effect and prevents overdestaining. This treatment should be kept as brief as possible. After reduction, the gel must be washed extensively. It is essential to wash out silver ions quantitatively before application of Farmer's reducer. Washing should therefore be tested with an HCI solution, which becomes turbid in the presence of Ag+ due to precipitation of AgCI. Farmer's reducer also must be completely washed out before recycling is started with the silver nitrate solution, as otherwise the gel may turn yellow or brown. Using a silver staining procedure, some proteins may react relatively weakly, especially with prior Coomassie Brilliant Blue staining. In that case, recycling of the silver staining is necessary, which also generates the desired maximal brown-black staining of the protein bands. Further recycling is possible, though not recommended, because an increase in

band staining can hardly be obtained without inducing background staining.

3.3 Sensitivity

Not only silver staining but also staining with dyes depends on different staining procedures including a variable and not always well-defined set of parameters. Therefore, comparison of a silver staining procedure with dye staining is not sufficient, because its quality varies with the method used. In order to compare the detection sensitivity of any staining method in polyacrylamide gels, the amount of protein per band which is just detectable should be specified. In addition to the staining method itself, the type of polyacrylamide gel (SDS gel or IEF gel) is of crucial importance. Whereas the detection limit of our silver staining method in a 1 mm thick SDS gel is about 0.5-1 ng of protein per band (Fig. 1a), an IEF gel (Fig. 2a) must contain 30-70 ng of protein per band in order to obtain a band stained with similar intensity, which means that the detection sensitivity for proteins is more than one-tenth higher in SDS gels than in IEF gels. This is not only observed with our silver stain but also with other staining methods using dyes such as Coomassie Brilliant Blue R-250 or G-250 and Amido Black 10 B.

Reference:
Heukeshoven J.: Simplified silver staining of proteins. Electrophoresis 1985, 6, 103-112