Thinking of Switching The Buffer Conditions Used In Your Protein Gel Runs? Think Again!

A wide variety of pre-cast polyacrylamide gels are now available from a number of suppliers. Different laboratories may choose different gel types with different buffer systems for similar applications which can affect the outcome of electrophoretic protein separations and the information obtained from one lab to the next. Why is this?

The mobility of a completely denatured protein in polyacrylamide gel electrophoresis (PAGE) in the presence of SDS is a function of its length. Nevertheless some proteins do not show their 'true' molecular weight, which can be calculated from its amino acid sequence, but instead present a deviating molecular weight which is called the apparent MW. In many cases this deviation can be detected with proteins which show a strong secondary structure and with peptides as well because the effect of secondary structure increases relative to the total size of the peptide. This phenomenon is caused by the maintenance of elements of secondary structure even in the presence of SDS (1).

Another observation is that a protein in different buffer systems for SDS PAGE shows different mobilities (2). In comparing two proteins this means that the difference in migration distance can be very high in a gel using one buffer system but very low in a gel using another buffer system. In extreme cases the pattern (order) of bands can be reversed. The explanation for this observation is that each buffer system has its specific pH which influences the electric charge of proteins and their binding capacity for SDS. For example, the following buffer systems are commercially available: Tris-Glycine (TG) (3), Tris-Tricine (TR) (4) and systems with neutral pH like VarioGel (5). Some gel types using these systems show very similar separation patterns of bands of a molecular weight standard although the gels differ in pH and concentration of polyacrylamide (Fig. 1).

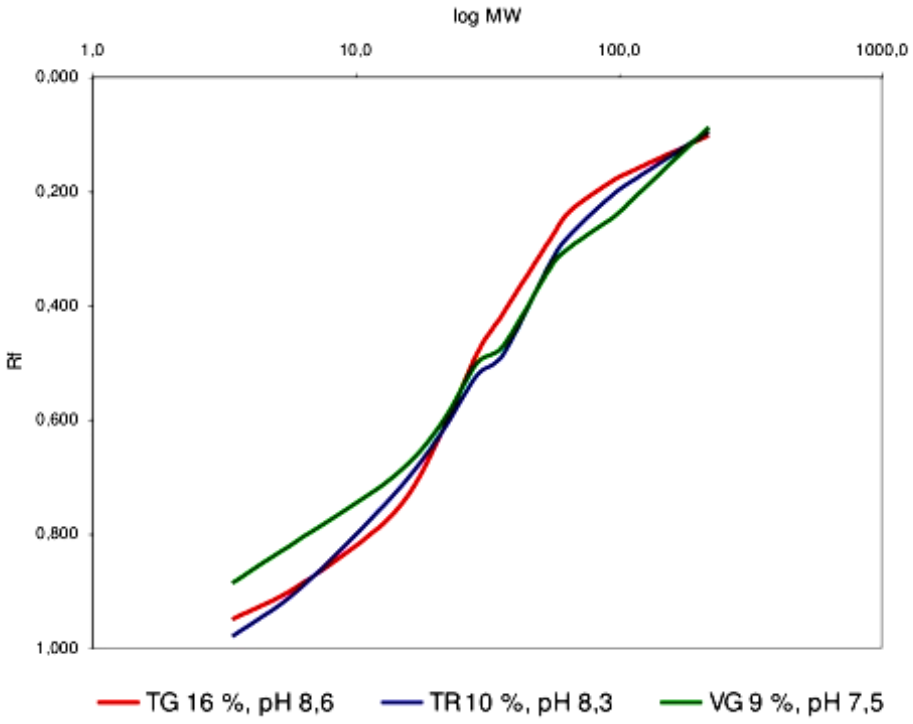

Fig. 1: Comparison of separation patterns of a molecular weight standard in gels of various buffer systems

If crude extracts from bovine muscle are separated in these gels and the resulting patterns compared distinct differences can be seen (Fig. 2). In all lanes there is a triplet of bands in the upper third of the gels and one strong band in the lower third. Above the triplet, band 1 and 2 can be seen in all gels. The distance between

these two bands is smallest in the TG gel whereas it is greatest in the VarioGel. On the other hand band 3 and 4 are clearly separated in the TG gel but they seem to appear as one band in the VarioGel. Intensity of staining of band 5 and 6 is different with the TG gel whereas it is equal in the TR and VarioGel. Mobility of band 7 and 8 differs mostly in the VarioGel whereas these two bands can not be separated in the TR system.

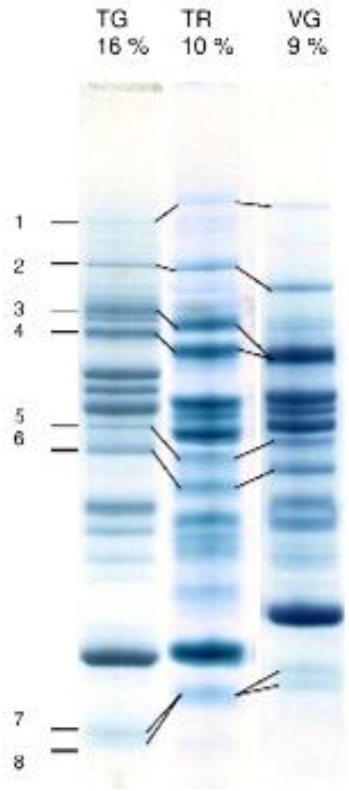

Fig. 2: Comparison of separation patterns of bovine muscle extract in gels of the buffer systems TG (Tris-Glycine-SDS), TR (Tris-Tricine-SDS), VG (VarioGel)

What does this all mean for optimal results in electrophoresis? If the mobility of a protein is a function of the above mentioned factors, predicting the best separation system is difficult. This prediction gets more difficult or almost impossible if several proteins or more complex protein mixtures have to be analysed. There is no single universal solution that meets all requirements needed for optimal results, instead the best solution for each specific application needs to be determined experimentally.

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