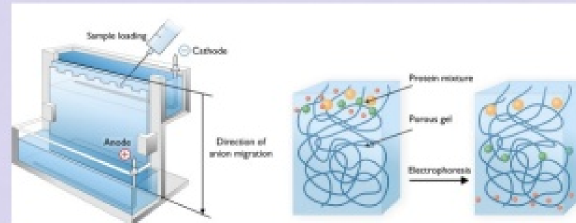
SDS PAGE

Analysis of Proteins

Background

SDS-PAGE is widely used to analyze the proteins in complex extracts. The most commonly used methods are derived from the discontinuous SDS-PAGE system first described by Laemmli (1970).
The system actually consists of two gels - a resolving (running) gel in which proteins are resolved on the basis of their molecular weights (MWs) and a stacking gel in which proteins are concentrated prior to entering the resolving gel. Differences in the compositions of the stacking gel, resolving gel and electrophoresis buffer produce a system that is capable of finely resolving proteins according to their MWs.



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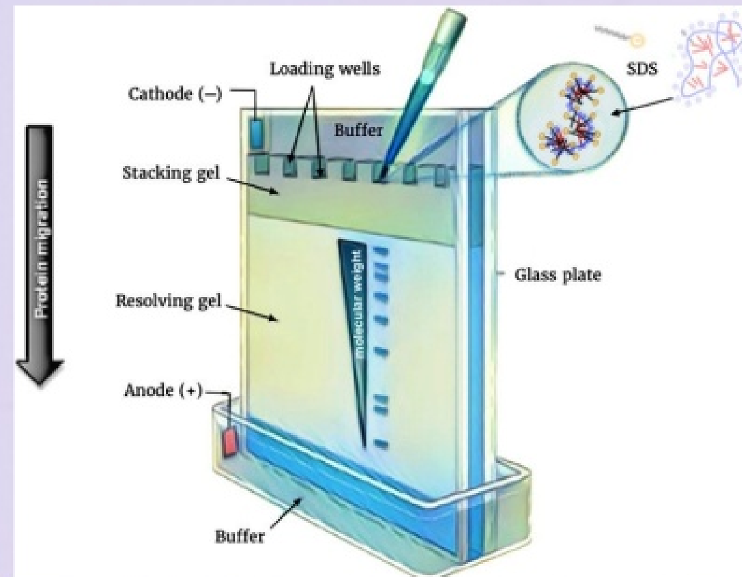
The principle



Gel electrophoresis

Chemistry of acrylamide

Protein denaturing

S&R Gels

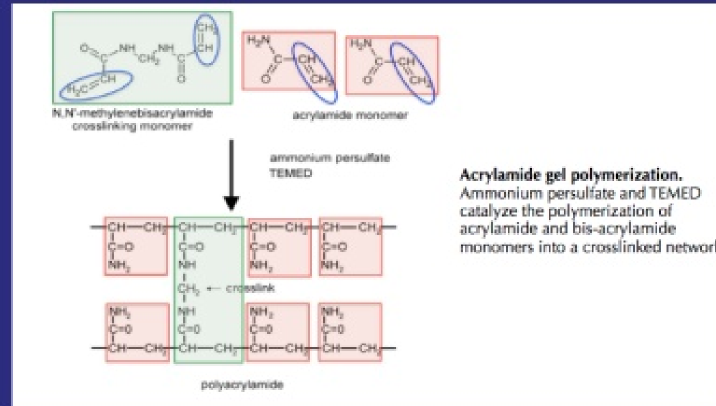
Gel electrophoresis of macromolecules

In gel electrophoresis, an electric field is used to move charged molecules through a matrix of a polymerized substance such as agarose or polyacrylamide. The rates at which individual molecules move through the gel depend on the properties of both the separation system and the molecules themselves. Gel matrices are permeated with networks of pores through which the molecules move. The amount of resistance that the matrix presents to the movement of a molecule depends on the diameter of the pore as well as the size and geometry of the molecule. Researchers can control the size of the pore by adjusting the concentration of gel monomer within a certain range. In general, smaller, more highly charged molecules migrate more rapidly through gels than larger or less charged molecules. The mobility of a molecule is also affected by the buffer system and the strength of the electrophoretic field used for the separation.

Chemistry of acrylamide polymerization

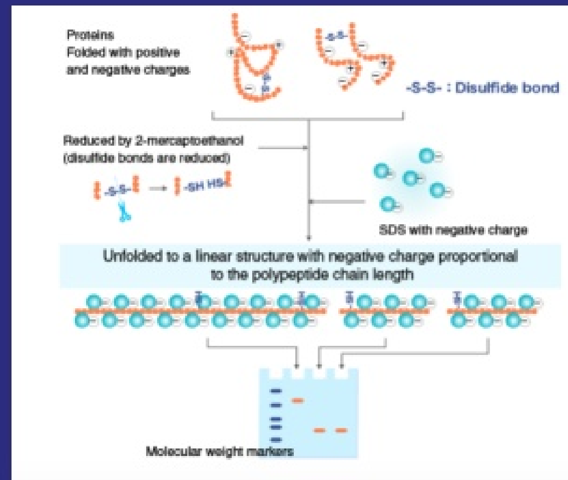
The polyacrylamide gels used to separate proteins are formed by the chemical polymerization of acrylamide and a cross-linking reagent, bisacrylamide{N,N-Methylenebis(acrylamide)} . Investigators are able to control the size of the pores in the gel by adjusting the concentration of acrylamide, as well as the ratio of acrylamide to bisacrylamide. Raising either the concentration of acrylamide or bisacrylamide, while holding the other concentration constant, this will decrease the pore size of the gel. Polymerization occurs because of free oxygen radicals that react with the vinyl groups in acrylamide and bisacrylamide, as shown in the figure below. The oxygen radicals are generated from the catalyst, ammonium persulfate (APS), when it reacts with a second catalyst, (TEMED).

Picture



Acrylamide gel polymerization. Ammonium persulfate and TEMED catalyze the polymerization of acrylamide and bis-acrylamide monomers into a crosslinked network.

protein-denaturing



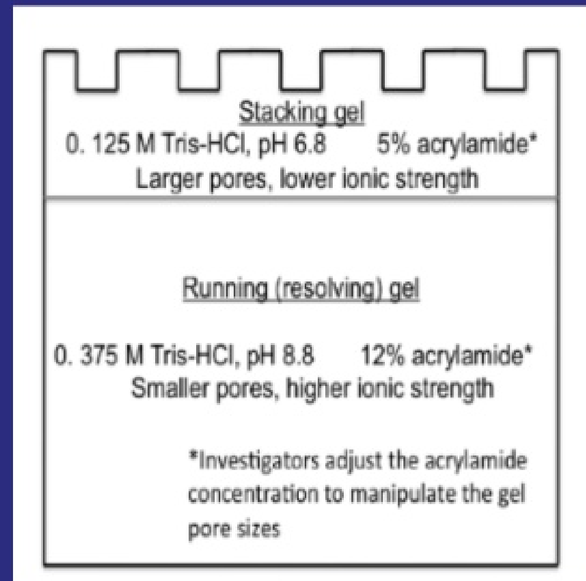
Discontinuities between the stacking and running gels underlie the resolving power of the SDS-PAGE gels

Step 1

Step 2

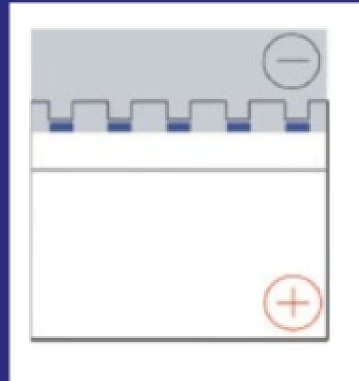
Step 3

Step 4


Stacking gel
0. 125 M Tris-HCl, pH 6.8
5% acrylamide*
Larger pores, lower ionic strength
Running (resolving) gel
0. 375 M Tris-HCl, pH 8.8
12% acrylamide*
Smaller pores, higher ionic strength
*Investigators adjust the acrylamide concentration to manipulate the gel pore sizes

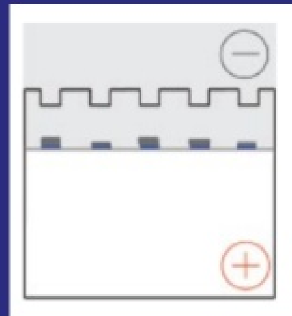
Step 2

The sample buffer used for SDS-PAGE contains a tracking dye, bromophenol blue (BPB), which will migrate with the leading edge of the proteins being separated on the gel. The gel is vertically positioned in the electrophoresis apparatus and covered with chamber buffer containing glycine



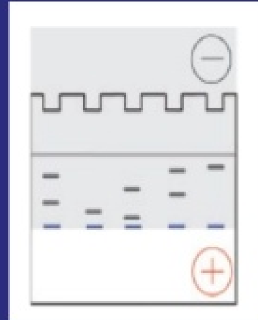
Step 3

Once a voltage is applied, the chloride ions in the sample buffer and stacking gel move rapidly toward the positive pole. The large pores of the stacking gel present very little resistance to the movement of protein-SDS complexes, which then "stack up" into a very concentrated region at the interface between the running and stacking gels .



Step 4

The pores in the running gel are much smaller than those of the stacking gel, so the pores present frictional resistance to the migration of proteins. Proteins begin to migrate at different rates, because of the sieving properties of the gel. Smaller protein-SDS complexes migrate more quickly than larger protein-SDS complexes. Within a certain range determined by the porosity of the gel, the migration rate of a protein in the running gel is inversely proportional to the logarithm of its MW



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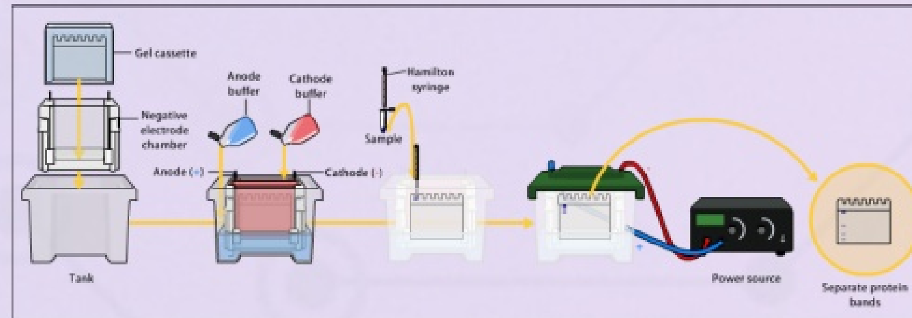
Procedure



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"People will forget what you said, people will forget what you did, but people will never forget how you made them feel"
~ Maya Angelou

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