1. Polyacrylamide Electrophoresis (PAGE) constant pH

In this method, the gel is produced by polymerising the acrylamide in the presence of a buffer solution. The polyacrylamide gel is formed by the monomer, acrylamide, the cross-linking agent, methylene-bisacrylamide, and a free radical generator, ammonium persulfate, in aqueous buffer. The polyacrylamide acts as a 3D scaffold, which supports an aqueous buffer solution. The pore size in polyacrylamide gels is controlled by the gel concentration and the degree of polymer cross-linking. Movement of charged species due to the application of an electric field through such a gel is proportional to the protein's NET charge (however frictional "drag" will oppose this movement). Thus the actual velocity will depend on (1) net charge (2) size (3) shape (4) electrical potential (5) ionic strength and pH of buffer (6) viscosity and temperature of medium ("gel"). It is a simple, rapid, and highly sensitive analytical tool for proteins and peptides.

PAGE is also used to separate relatively small oligonucleotides

2. Iso-electric focussing with PAGE (IEF) gradient pH

When the pI of a protein = pH of buffer, there is zero net charge and then there will be no movement. IEF utilises a gel made by polymerising the acrylamide in the presence of a MIXTURE buffer solutions ("ampholytes"). An initial application of an electrical potential will set up a pH gradient across the gel. The protein mix is then applied and the electrical potential applied again. At the pI of a protein = pH of buffer, then there will be no movement, so proteins will move until they arrive at the section of the gel where their pI=pH and stops!

3. SDS Polyacrylamide Electrophoresis (SDS/PAGE)

In most cases, SDS binds to proteins in a constant ratio of 1.4 grams of SDS per gram of protein. On average, the number of bound SDS molecules is half the number of amino acid residues in the polypeptide. The negative charge due to SDS is much more than the negative and positive charges of the amino acid residues. The large quantity of bound SDS efficiently masks the intrinsic charges in the protein. Consequently, SDS denatured proteins are net negative and since the binding of the detergent is proportional to the mass of the protein, the charge to mass
ratio is constant. In addition, the shapes of SDS denatured proteins are the same (rodlike). The size of the rod-like chains is the only gross physical difference between SDS denatured proteins. The larger the molecular weight of the protein the longer the rod-like chain. The pores in the gel distinguish these size differences. During SDS electrophoresis, the proteins migrate through the gel towards the positive electrode at a rate that is inversely proportional to their molecular weight. In other words, the smaller the protein, the faster it migrates. The molecular weight of the unknown is obtained by the comparison of its position after electrophoresis to the positions of standard SDS denatured proteins electrophoresed in parallel. The relative molar masses RMM/molecular weights of the standard proteins have been previously determined with great accuracy. After the proteins have been visualized by staining the gel with dyes, their migration distance is measured. The log10 of the molecular weights of the standard proteins are plotted versus their migration distance. Taking the logarithm or the Rf allows some of the data to be plotted as a straight line. The molecular weight of the unknown is then easily calculated from the standard curve.

The electrophoretic mobility of the proteins is also affected by the gel concentration ("pore size") due to cross-linking. Higher percentage gels are more suitable for the separation of smaller proteins and of peptides. Polyacrylamide gels can be prepared to have a gradient of gel concentrations. Typically the top of the gel (under the sample wells) has a concentration of 5%, increasing linearly to 20% at the bottom. Gradient gels can be useful in separating protein mixtures that cover a large range of molecular weights. Gels of homogeneous concentration are better for achieving wider separations of proteins that occupy narrow ranges of molecular weights.

4. Agarose Electrophoresis:

This uses a different medium, agarose, made from a polysaccharide and is used for the electrophoresis of nucleic acids. This is the same material used in size exclusion chromatography (SEC/GPC/GFC). The pore size is much larger than PAGE and is only used for polynucleotides and very large proteins.

5. Electrophoresis of Blood Plasma Proteins

Blood plasma is thought to contain over 100 different proteins. Both PAGE and SDS/PAGE are useful methods for the fractionation and analysis of these proteins: in clinical tests agarose GE is usually used. It
has been observed that the plasma proteins can be separated into 6 "bands" each containing one or more proteins. Patient data is compared to "reference" data. Pattern recognition is used to estimate the presence of excess or decreased protein in a particular band. These changes have been correlated to specific disease.

The most darkly staining band of the plasma proteins is due to albumin, with a molecular weight of approximately 68,000. It is directly under the transferritin band. Albumin is the most abundant plasma protein and is one of the few that is not a glycoprotein. It consists of a single polypeptide chain with 17 interchain disulfide bonds. As in the immunoglobulins, the disulfide links help the albumin fold into three structural domains, each consisting of three sub-domains. These domains form the wide variety of ligand binding sites found in the protein. Albumin functions in the binding and transport of fatty acids, Ca$^{2+}$, Ni$^{2+}$, bilirubin, tryptophan, steroid hormones, and many drugs such as sulfonamides, penicillin and aspirin. A major physiological role of albumin is in osmotic regulation. The protein is responsible for 80% of the osmotic properties of plasma since it is more than half the plasma proteins by weight, has the lowest molecular weight of the major proteins and contains 18 negative charges at physiological pH. The charge effects the distribution of sodium and chloride ions in the extracellular fluids and consequently, plasma osmolarity. Below the albumin are several partially resolved darkly stained bands, between the 67,000 and 43,000 markers. The heavy chain family of the immunoglobulins (mostly IgG subclasses) migrate in this region.