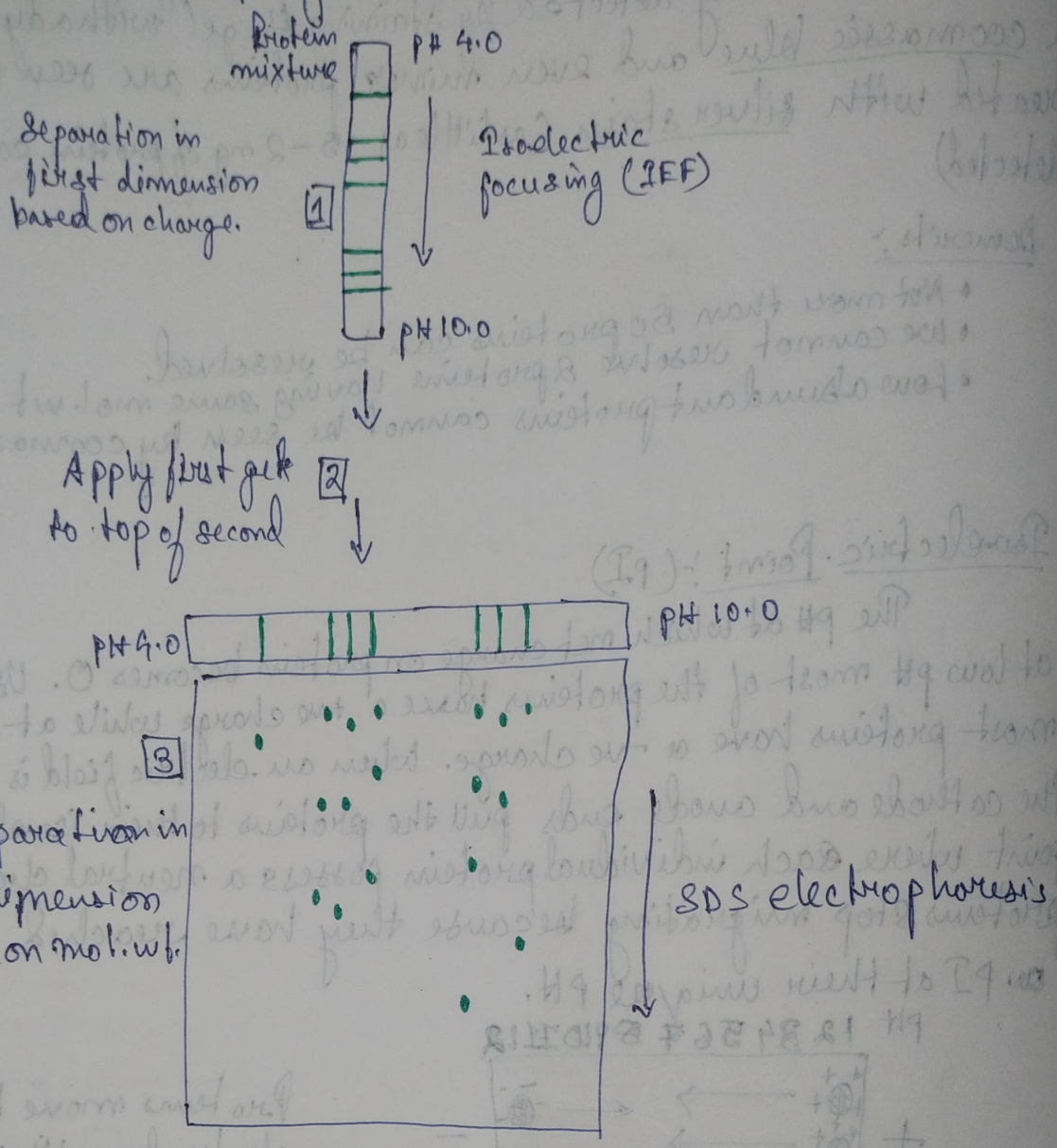
2D-PAGE

Immobilised pH gradient strip - IPG strip



Two-dimensional polyacrylamide-gel electrophoresis:-

Since proteins with similar size tend to overlap, 1-D separational methods, such as SDS-PAGE or chromatography, can resolve only a relatively small no. of proteins (usually less than 50). In contrast 2D gel electrophoresis (2-DE), which combines 2 diff. procedures can be used to resolve more than 1000 proteins in the form of a 2D protein map. In the first step proteins are separated on the basis of their intrinsic charge. The protein samples are

dissolved in a small vol. of sol. containing a non ionic (uncharged) detergent together with 2 mercapto ethanol and denaturating agent urea. The solution solubilize, denatures and dissociates all the polypeptide chains and leave their intrinsic charge unchanged. The pp are then separated by IEF, which depends on the fact that net charge on the protein molecules varries with pH of the surrounding sol. For any protein their is a characteristic pH called its isoelectric point (PI), at which the protein has no net charge and ∴ will not migrate in an electric field. In isoelectric focusing proteins are electrophoresed in a narrow tube of polyacrylamide gel in which a gradient of pH is established by mixing special buffers. Each protein moves to a position in gradient that corresponds to its isoelectric point and stays their. This is the 1st dimension of 2DE. (Fig. of PI)

In the 2nd step the narrow gel containing the separated proteins (based on their charge) is again subjected to electrophoresis but in a direction at rt. angle to that used in the 1st step. This time SDS is added and the proteins are separated according to their size just 1D - SDS PAGE. The original narrow gel is soaked in SDS and then placed on one edge of SDS gel slab through which each polypeptide chain migrates to form a discrete spot. This the 2nd dimension of 2DE. The only proteins left unresolved will be those that have both an identical size and identical PI, a relatively rare situation. Even trace amount of each pp. chain can be detected on the gel by various staining procedure or autoradiography if the protein sample of was initially

labelled with radioisotope. The resolving power of 2DF is so high that 2 protein differ in ~~eight~~ single charged AA can be readily resolved.