Clinical Chemistry

Disc Electrophoresis

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A species of techniques have been used in clinical chemistry laboratory for sample testing

Most Fundamental Methods Include:

Electrophoresis
Chromatography
Spectrophotometry
Mass spectrometry
Fluorometry
Nephelometry
Turbidimetry
Biochip(Protein and DNA Chip/Array)
Biosensor

Disc Electrophoresis

Serum protein zones determined by ordinary electrophoretic techniques contain several proteins with the same electrophoretic mobility and they tend to be large because proteins diffuse during electrophoresis. Disc electrophoresis was introduced in 1964 to overcome these deficiencies.

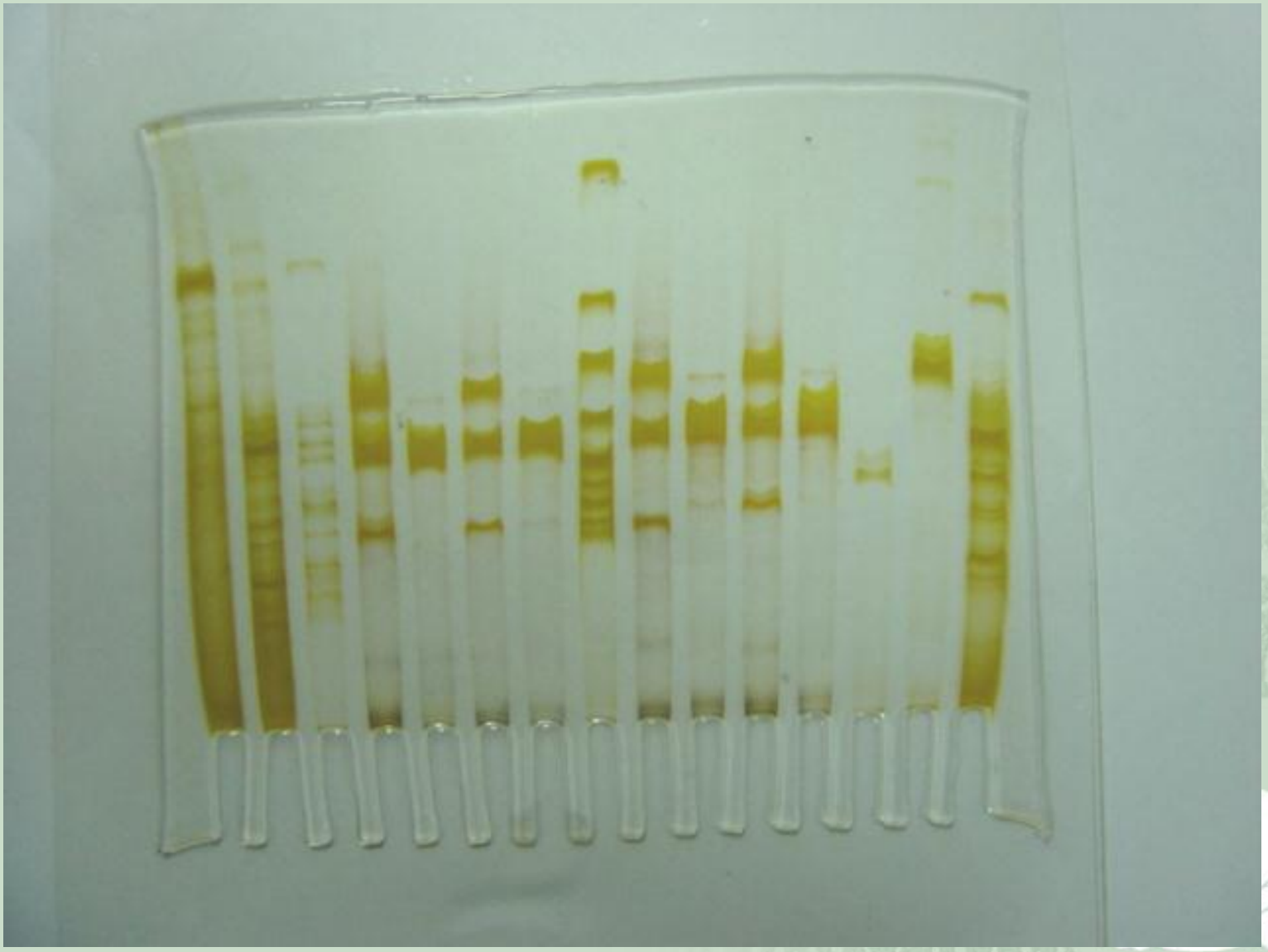
Polyacrylamide Gel Electrophoresis (PAGE)

In this technique, individual gels are prepared in glass tubes by polymerizing a gel monomer and a cross-linking agent with the aid of an appropriate catalyst. The first gel to be poured into the tubular-shaped electrophoresis cell is the small-pore separation gel . After 30 min, during which gelation takes place, a large pore gel, *the spacer Gel* is thrown on top of the separation gel.

Then a large-pore monomer solution containing a small amount of sample (serum) is polymerized above the spacer gel so that the finished product is composed of three different layers of gel.

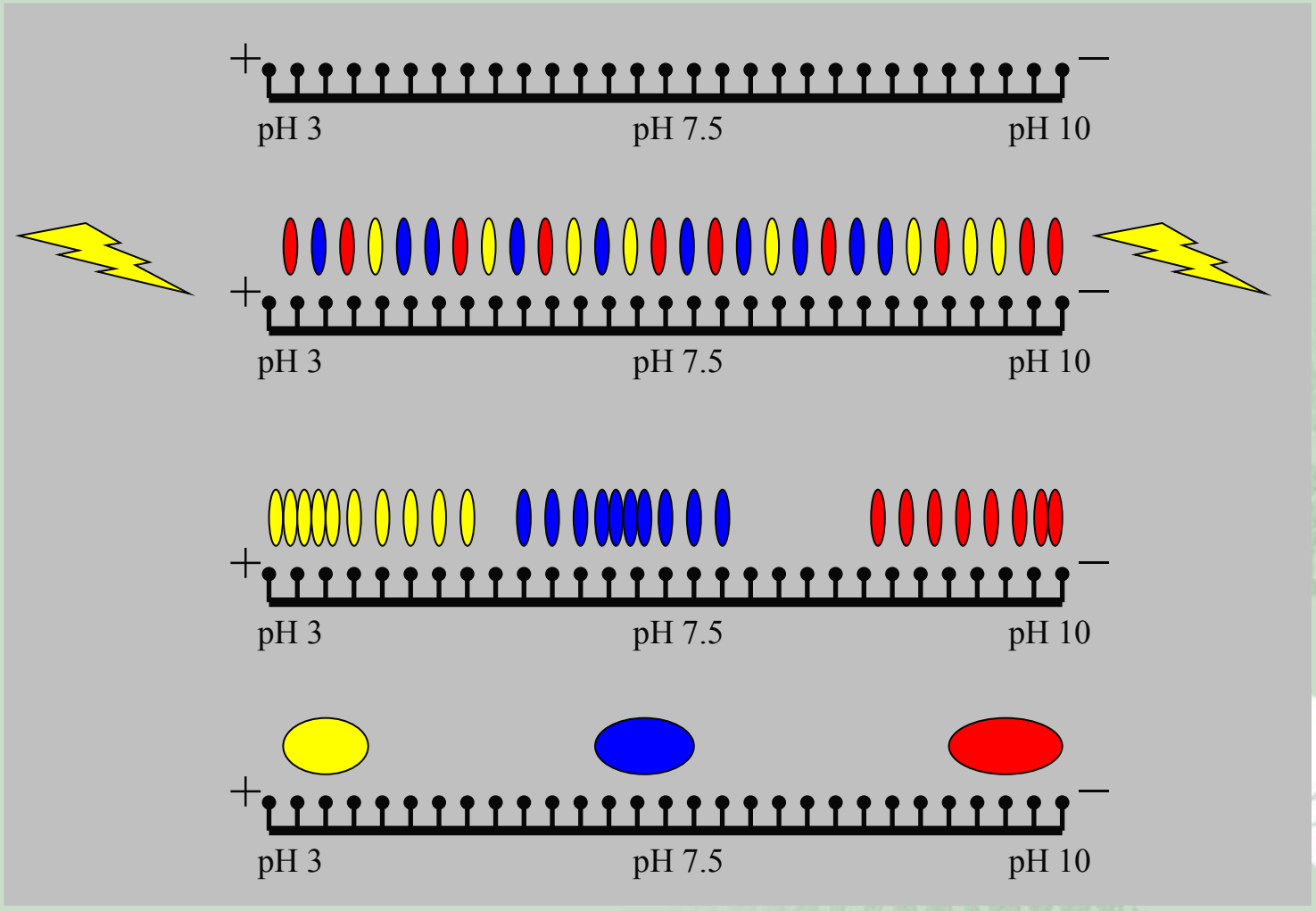
When electrophoresis begins, all protein ions migrate through the large-pore gel and amass on the separation gel in a very fine zone.

This process improves the resolution and concentrate protein components at the border zone, so that pre-concentration of specimens with low protein content may not be necessary. Separation then takes place in the bottom separation gel with the retardation of some proteins due to the molecular sieve phenomenon.



Isoelectric Focusing (IEF) Electrophoresis

Amphoteric compounds , such as proteins, can be separated by virtue of migration in a medium possessing a stable pH gradient using isoelectric focusing (IEF) . The protein moves to a zone in the medium where the pH is equal to its isoelectric point (pI). At this pH, the charge becomes zero and migration ceases.


+
−
pH 3
pH 7.5
pH 10
+
−
pH 3
pH 7.5
pH 10
+
−
pH 3
pH 7.5
pH 10
+
−
pH 3
pH 7.5
pH 10

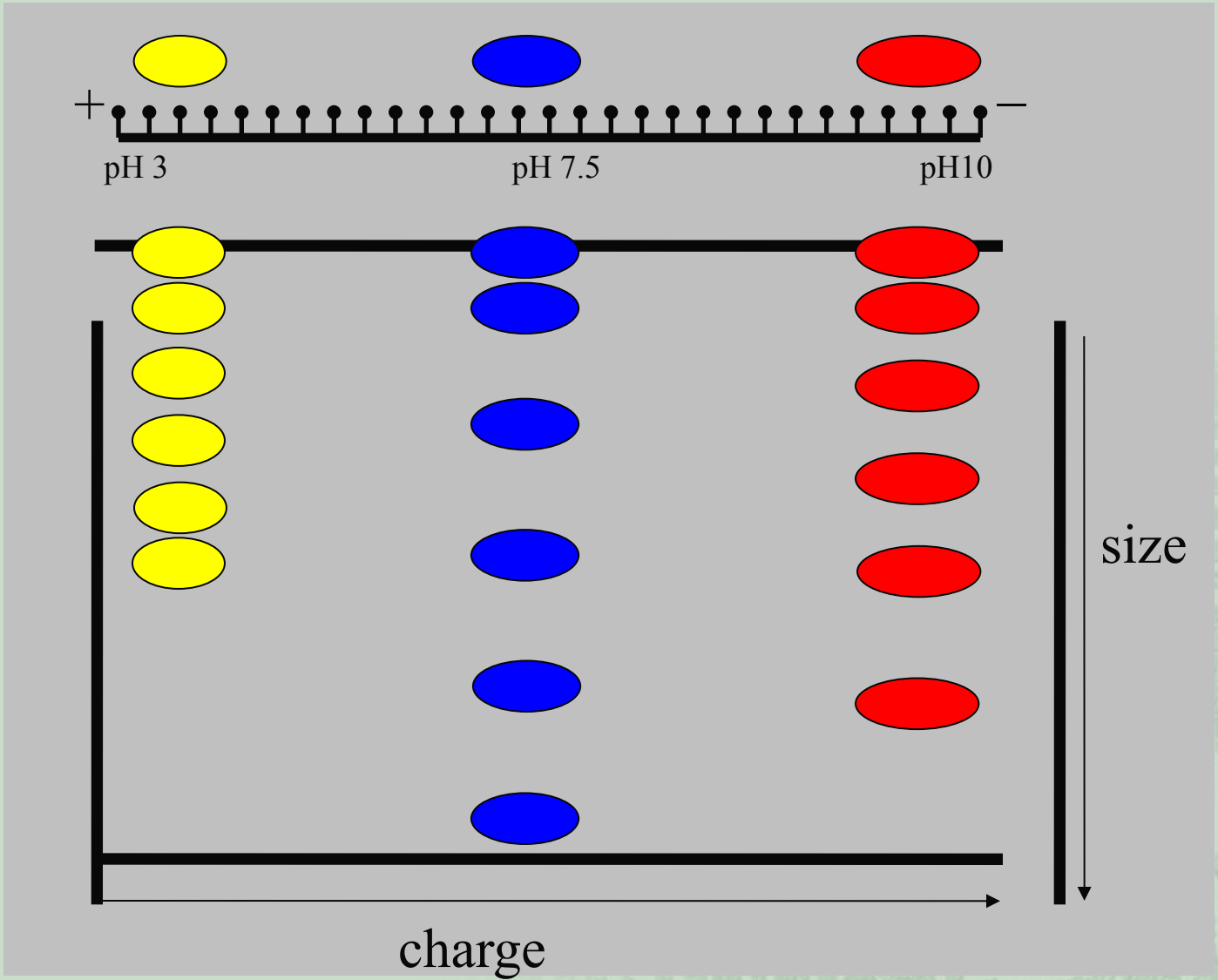
Two-Dimensional (2D) Electrophoresis

In the first dimension, it uses charge-dependent IEF electrophoresis in a large-pore medium such as agarose or large-pore polyacrylamide gel; and in the second dimension, molecular weight-dependent electrophoresis in polyacrylamide.

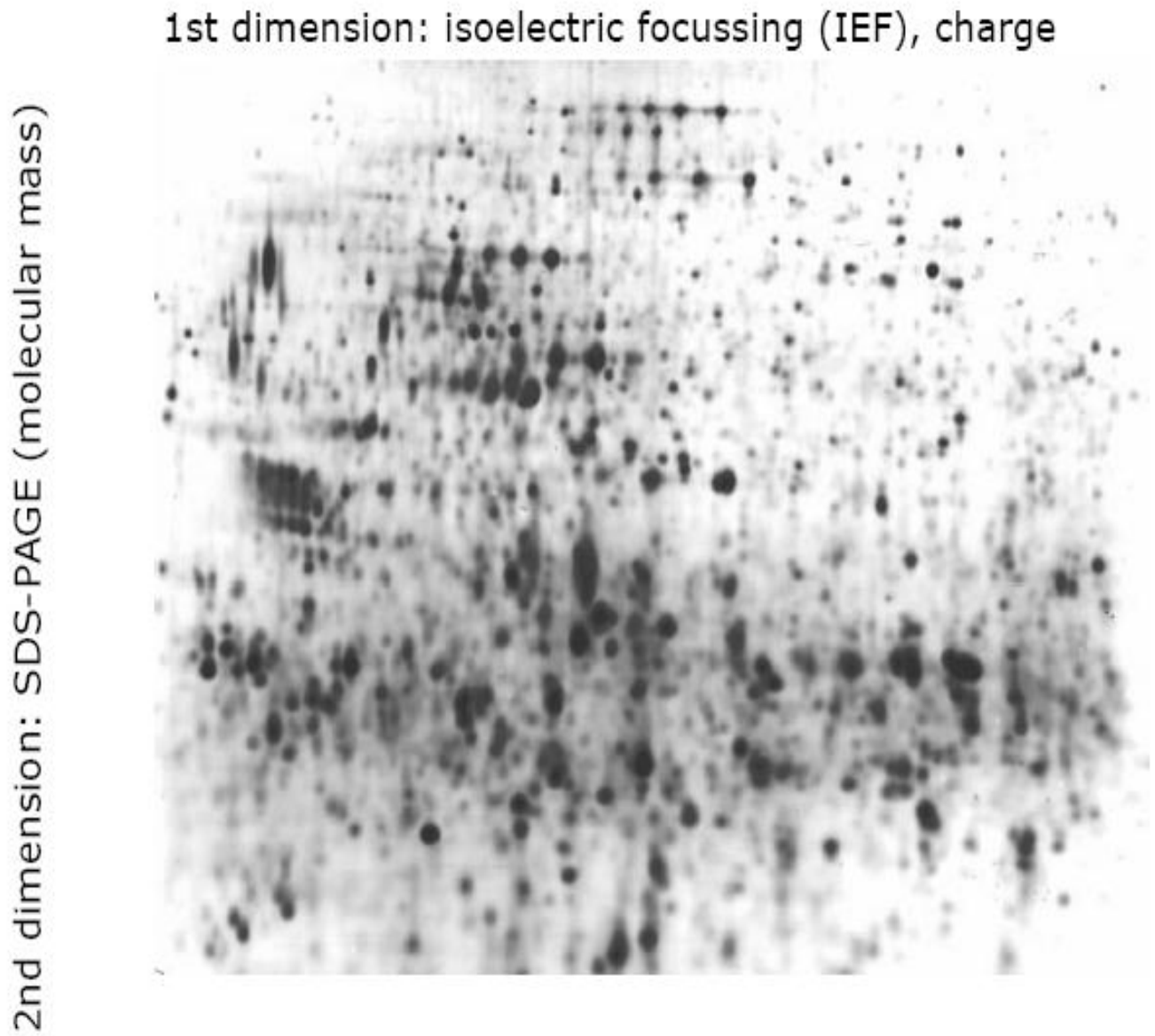
: SDS-PAGE

Proteins migrate through the gel at a rate proportional to their size.

Smallest proteins travel the furthest distance



2-dimensional polyacrylamide electrophoresis (2D-PAGE)



Capillary Electrophoresis (CE)

In CE, the classic techniques of electrophoresis are carried out in a small-hollow (10 - 100μm of diameter) fused silica capillary tube of 20 to 200 cm in length. This capillary tube is connected to a detector at its terminal end and, via buffer reservoirs to a high-voltage power supply

Two distinct advantages of the capillary format include the ease of automation and the efficient heat dissipation that permits the application of much high voltages (25 to 30 kV) than in traditional electrophoresis. This high voltage enhances separation efficiency and reduces separation time in some cases to less than 1 min.

2 - CHROMATOGRAPHY

Chromatography is a physical method of separation in which the components to be separated are distributed between two phases , one of which is stationary (stationary phase) while the other (mobile phase) moves in the fixed direction.

Basic concepts and definitions

The primary goal of the chromatographic process is to separate a mixture into its individual components , which are called solutes or analytes.

A chromatographic separation requires a sample to be introduced into a flowing stream of gas or liquid (mobile phase) that passes through a bed, layer, or column containing the stationary phase.

If the mobile phase is a gas, the technique is known as *gas chromatography* (GC) ,

if a liquid, it is called *liquid chromatography* (LC).

The stationary phase may be particles of a solid or gel or a liquid.

As the mobile phase carries the sample through the stationary phase, the solutes with lesser affinity for the stationary phase remain in the mobile phase and travel faster and separate from those that have a greater affinity for it.