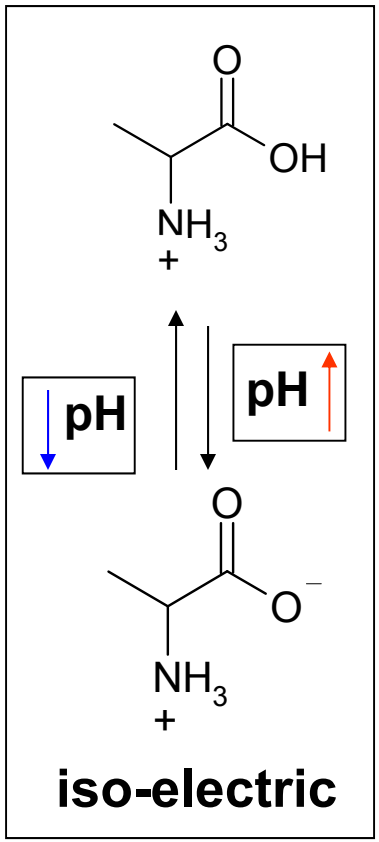
A. Modes of separation capillary electrophoresis

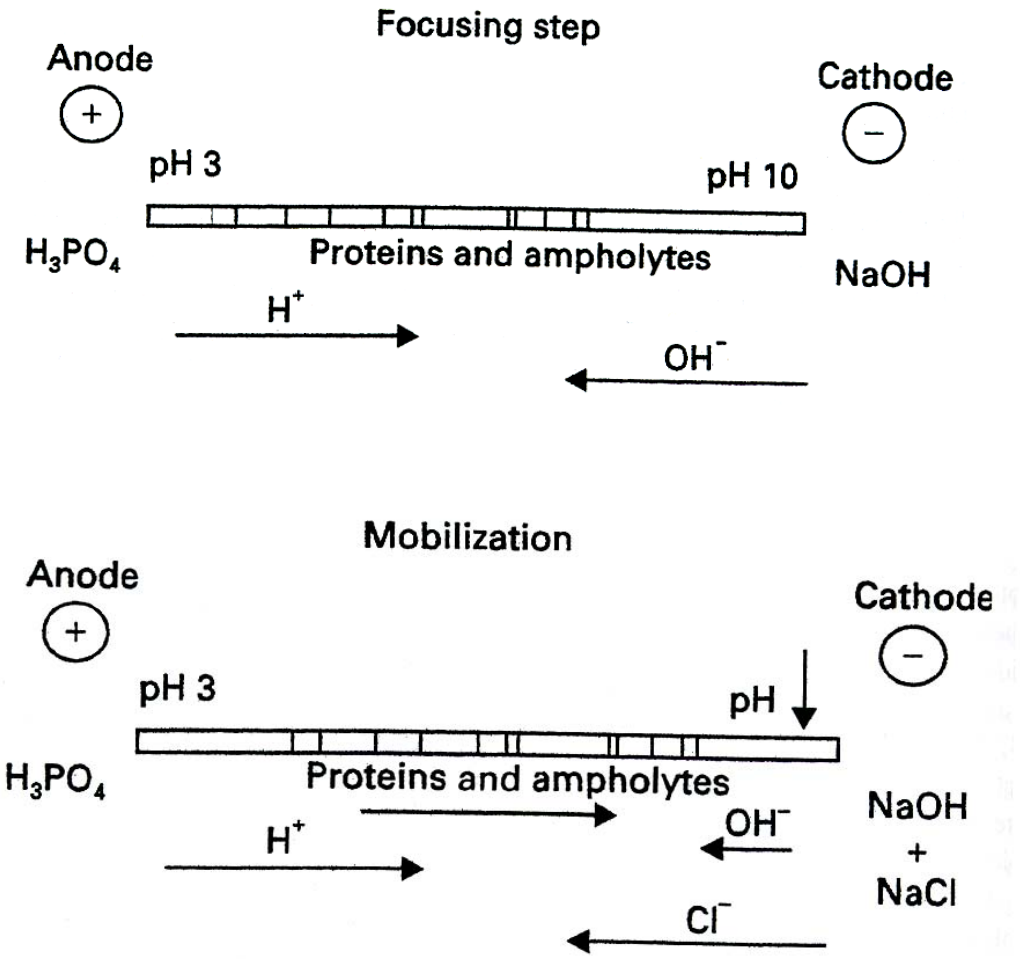
1. Capillary Zone electrophoresis
2. Capillary iso-electric focusing
3. Micellar electrokinetic Capillary chromatography
4. Capillary electrochromatography
5. Capillary gel electrophoresis

B. Electrophoresis for Bio-Applications

DNA, RNA, and protein

Capillary iso-electric focusing



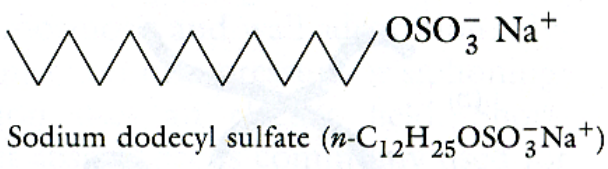


In this case, the electroosmotic force is weaker than elctrophoretic force.

Micellar electrokinetic Capillary chromatography

Separation of neutral solute

Psuedo-stationary phase



Sodium dodecyl sulfate (n-$C_{12}H_{25}OSO_3^-Na^+$)

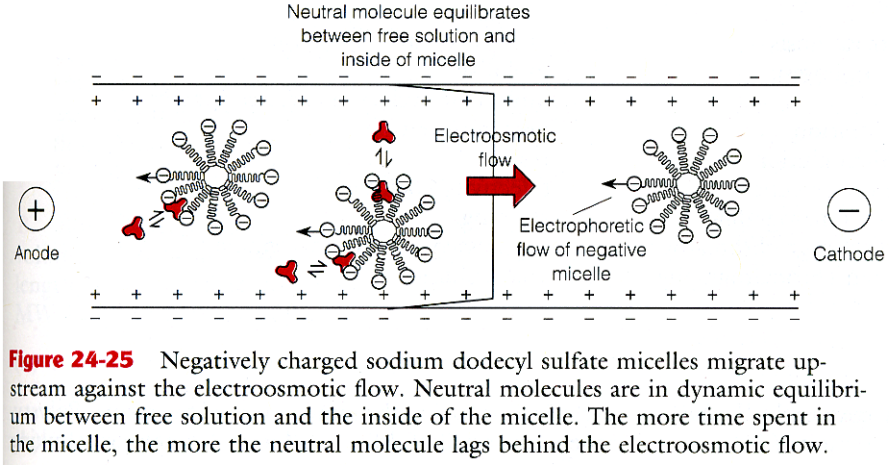
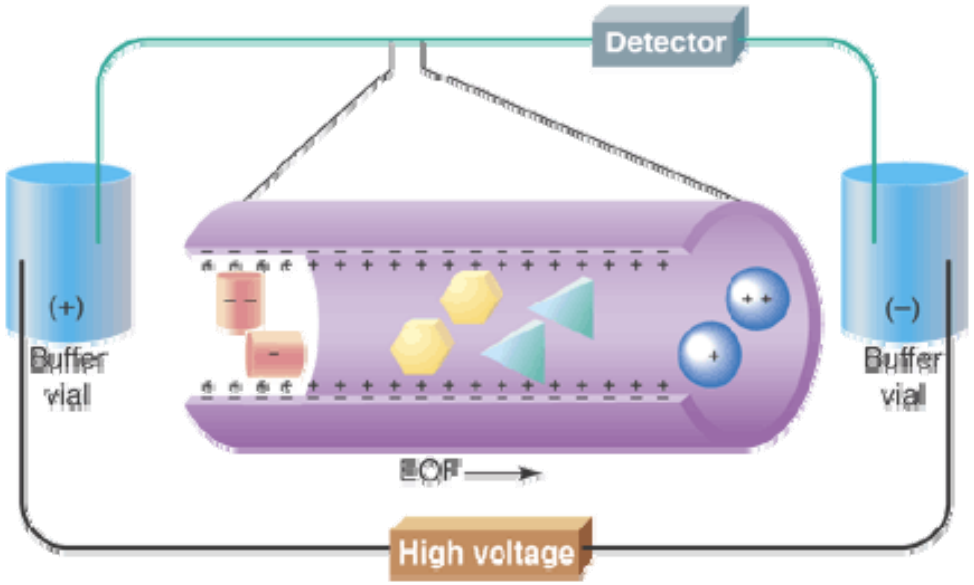


Figure 24-25 Negatively charged sodium dodecyl sulfate micelles migrate upstream against the electroosmotic flow. Neutral molecules are in dynamic equilibrium between free solution and the inside of the micelle. The more time spent in the micelle, the more the neutral molecule lags behind the electroosmotic flow.

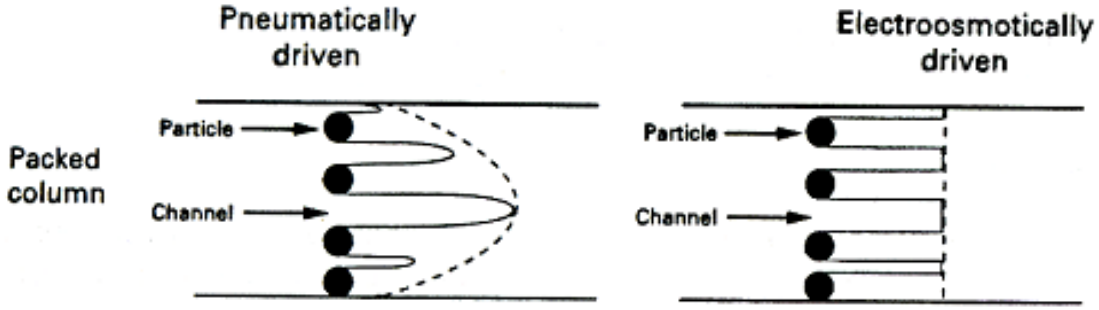
Advantage: easy to apply

Disadvantage: less selectivity



Capillary electrochromatography

Capillary electrochromatography is an electroosmotically driven liquid chromatographic technique.



	Stationary phase	Mobile phase
Capillary electrochromatography:	yes	yes
Capillary electrophoresis:	no	yes

	Charged solutes	Neutral solutes
Capillary electrochromatography:	yes	yes
Capillary electrophoresis:	yes	no

Capillary Electrochromatography: analysis of polycyclic aromatic hydrocarbones

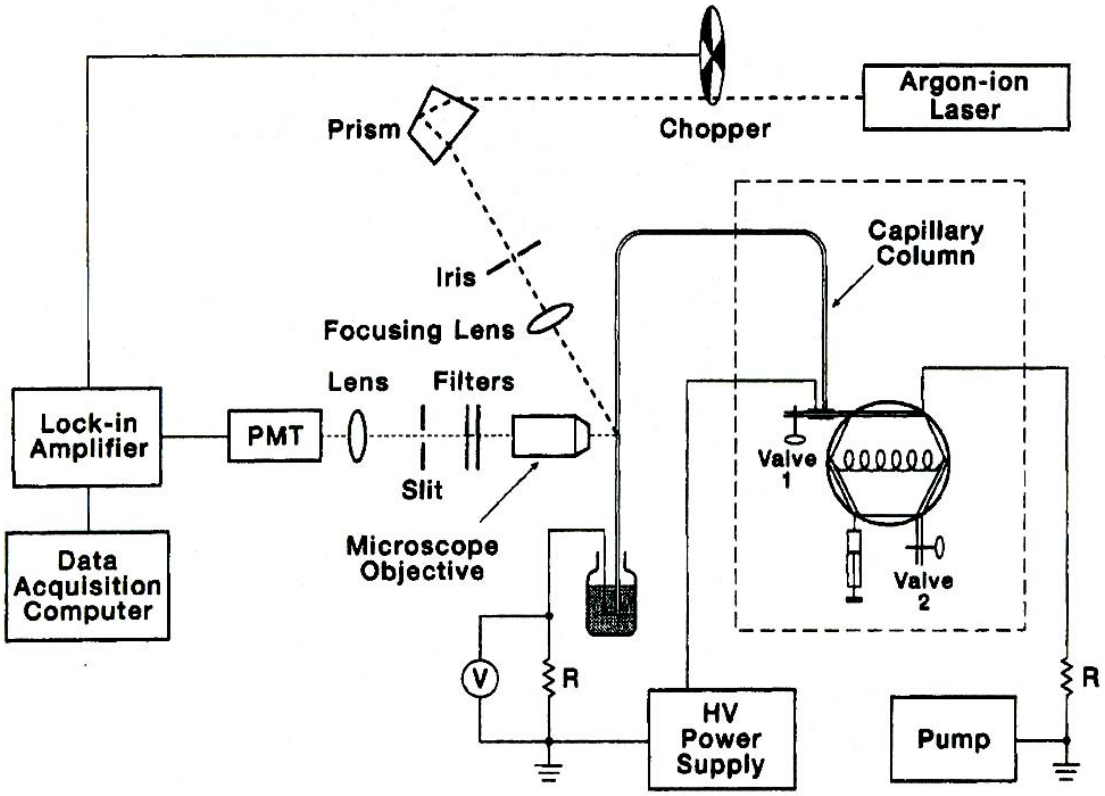


Figure 1. Schematic of the CEC-LIF apparatus.

Stationary phase: 90% 3-μm octyldecyl-silica particles; 10% 1-μm silica

Partition stationary phase

Stabilization of Electroosmotic flow

Mobile phase: mixture of acetonitrile and 4mM soldium tetraborate solution

R. N. Zare, et al., Anal. Chem. 1995, 67, 2026

Capillary Electrochromatography: analysis of polycyclic aromatic hydrocarbones

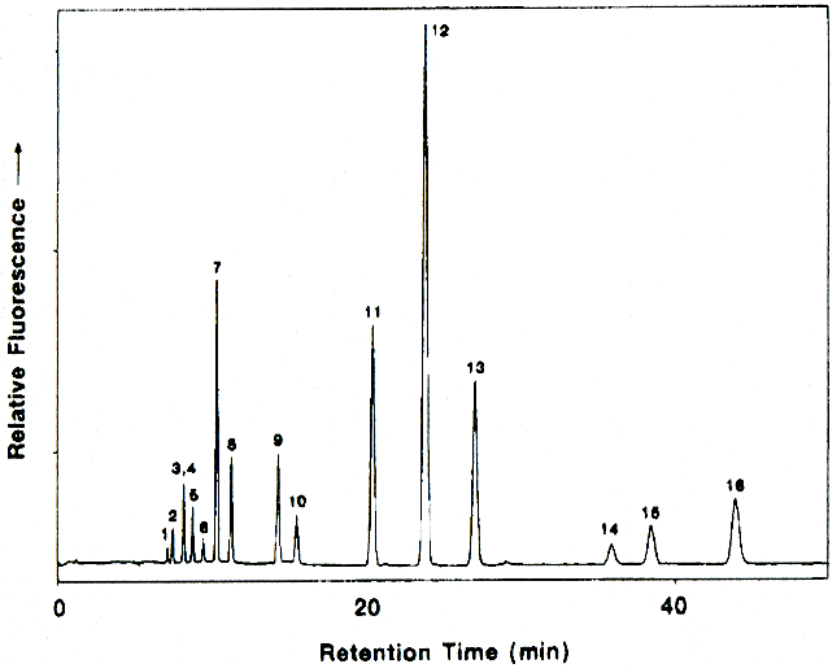


Figure 2. Electrochromatogram showing the capillary electrochromatographic separation of the 16 PAHs. The column dimensions were 75 μm i.d. × 365 μm o.d. (33-cm packed length). The mobile phase consisted of 80% acetonitrile in a 4 mM sodium borate solution. The applied voltage was 15 kV. Injection was performed electrokinetically at 5 kV for 5 s. The peaks are identified as follows (~10^{-6}–10^{-8} M of each compound): (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benz[*a*]anthracene, (10) chrysene, (11) benzo[*b*]fluoranthene, (12) benzo[*k*]fluoranthene, (13) benzo[*a*]pyrene, (14) dibenz[*a,h*]anthracene, (15) benzo[*ghi*]perylene, and (16) indeno-[1,2,3-*cd*]pyrene.

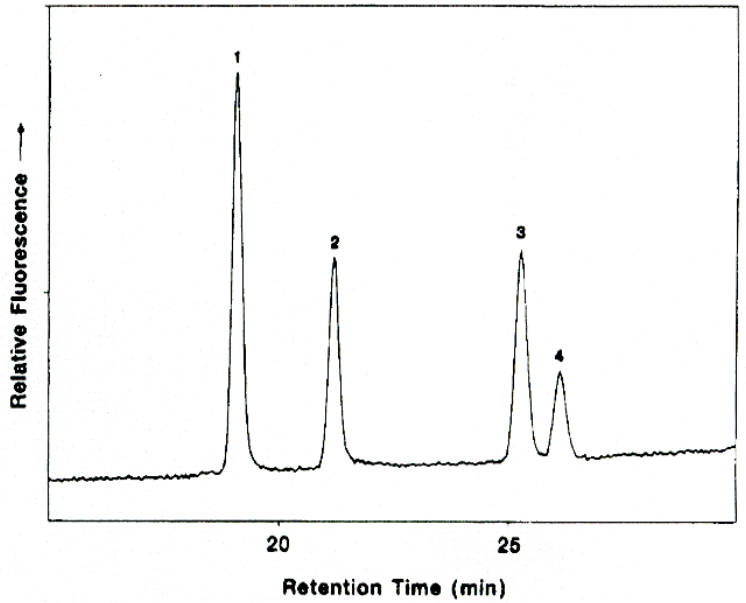


Figure 3. Electrochromatogram showing the separation of the first four PAHs. The conditions are the same as in Figure 2, except that the acetonitrile in the mobile phase has been changed to 60%.

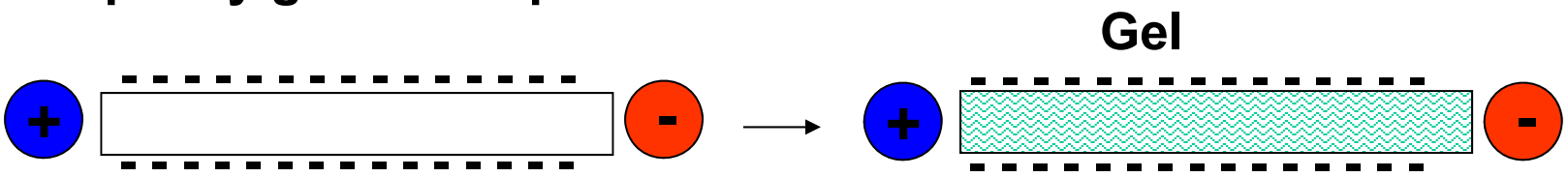
Table 2. Comparison of Efficiencies between CEC and Micro-HPLC

PAH	no. of theoretical plates (N)/m	
	CEC	micro-HPLC
naphthalene	102 000	67 000
fluoranthene	132 000	85 000
benz[*a*]anthracene	137 000	89 000
benzo[*k*]fluoranthene	138 000	103 000

$$N = 5.44\,(t_R/w_h)^2 = 16\,(t_R/w_b)^2$$

R. N. Zare, et al Anal. Chem. 1995, 67, 2026

5. Capillary gel electrophoresis



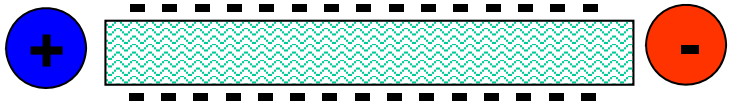
a. Blocking the solute diffusion caused by Joule heating

b. Size of the channels in the gel gives further selection (entropy effect)

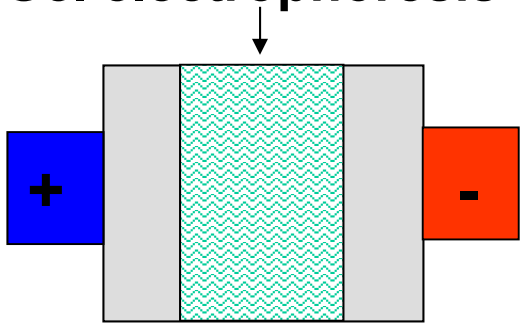
B. Electrophoresis for Bio-Applications

Separation of DNA, RNA, and protein

Capillary gel electrophoresis



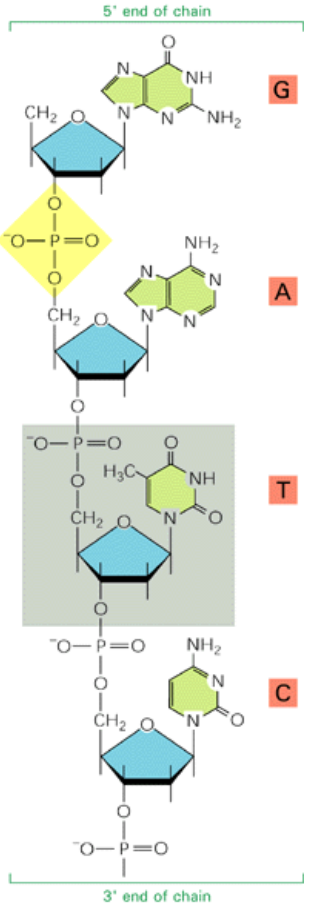
Gel electrophoresis



Electroosmosis can play a significant role in capillary gel electrophoretic separation, but not in gel electrophoretic separation.

Both techniques separate solutes by their eletrophoretic mobility.

Separation of DNA and RNA



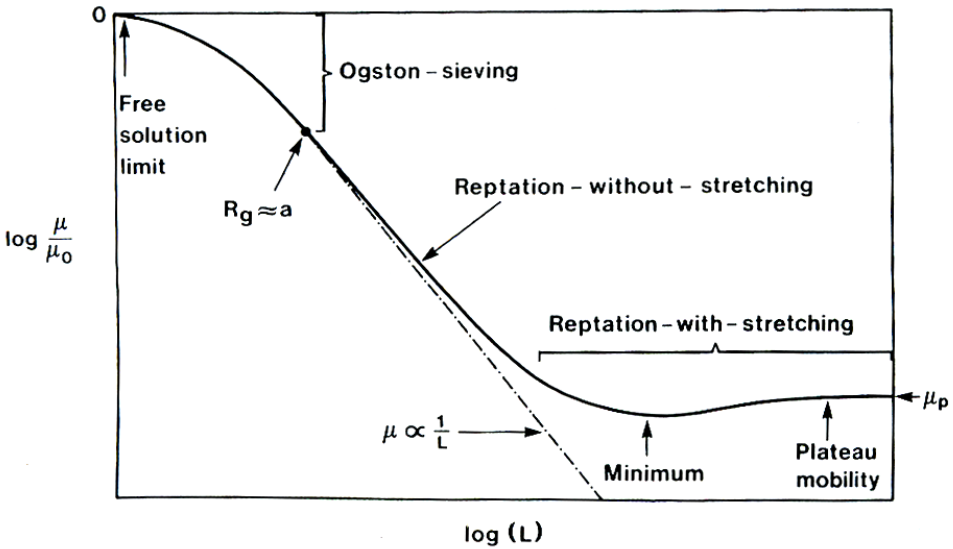
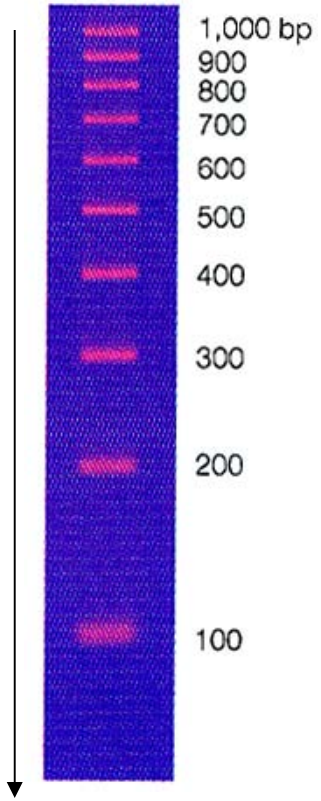
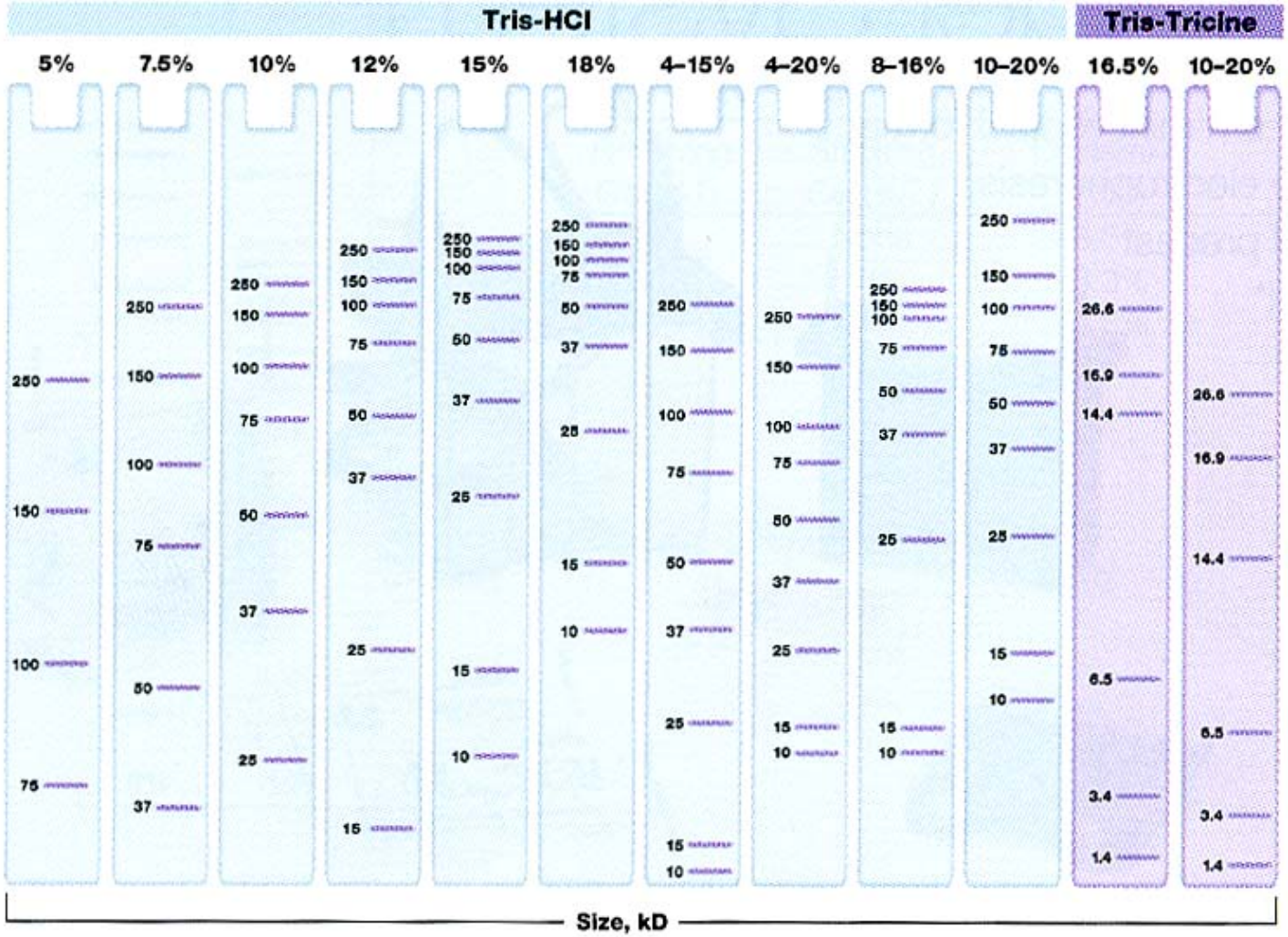


Figure 2. A schematic log-log plot of reduced mobility, μ/μ_0, *vs.* molecular size L. The various regimes are discussed in Section 2. From [23], with permission.



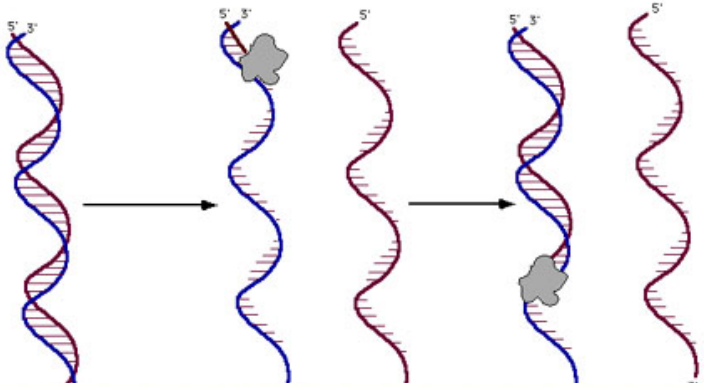
$$\mu \propto \frac{1}{L}$$

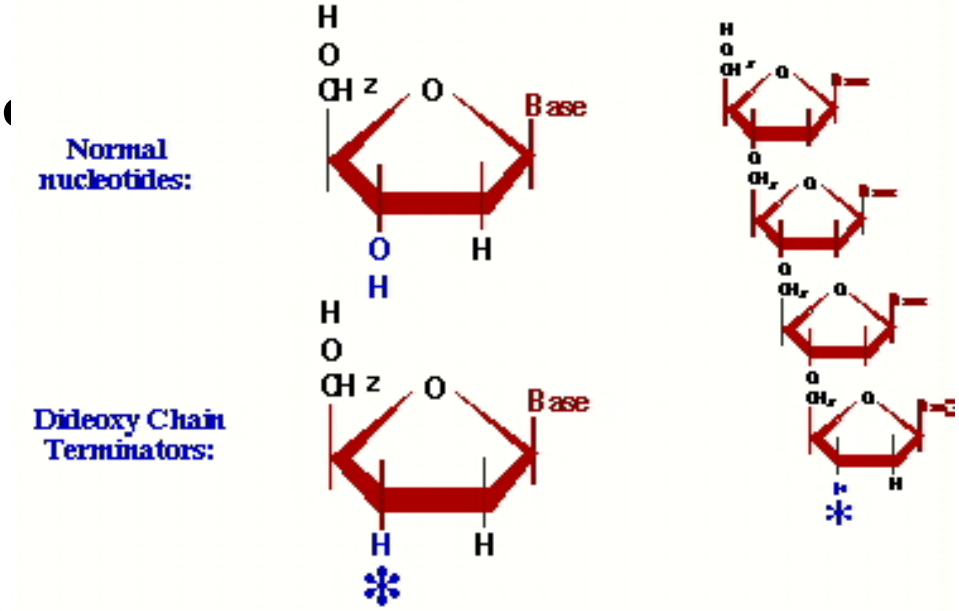
Separation of Protein

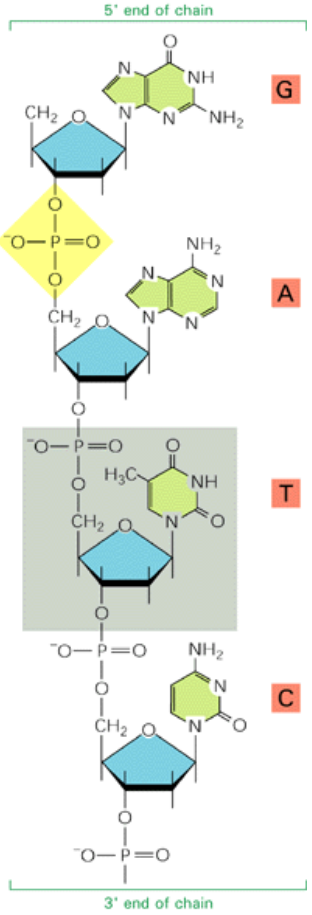


Size effect and electrophoretic mobility

Further applications
-- an example: DNA sequencing







Polymerase Chain Reaction (PCR)

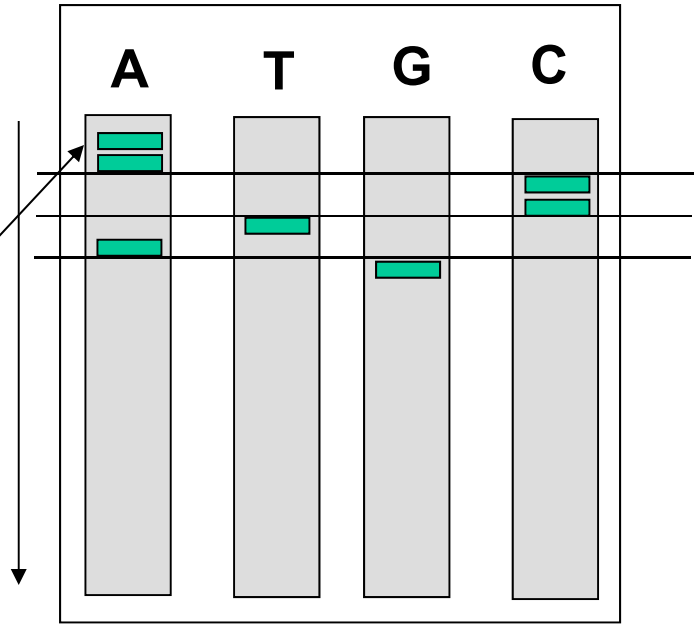
DNA Polymerase reads the template strand and synthesizes a new second strand to match:

```
5' - TACGCGGTAACGGTATGTTCGACCGTTTAGCTACCGAT
3' - ATGCGCCATTGCCATACAAGCTGGCAAATCGATGGCTAGAGATCCAA - 5'
```

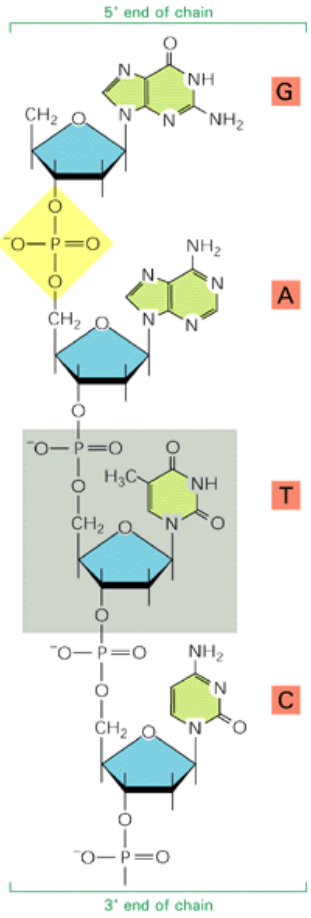
IF 5% of the T nucleotides are actually dideoxy T, then each strand will terminate when it gets a ddT on its growing end:

```
5' - TACGCGGTAACGGTATGTTCGACCGTTTAGCTACCGAT•
5' - TACGCGGTAACGGTATGTTCGACCGTTTAGCT•
5' - TACGCGGTAACGGTATGTTCGACCGTTT•
5' - TACGCGGTAACGGTATGTTCGACCGTT•
5' - TACGCGGTAACGGTATGTTCGACCGT•
5' - TACGCGGTAACGGTATGTT•
5' - TACGCGGTAACGGTATGT•
5' - TACGCGGTAACGGTAT•
5' - TACGCGGTAACGGT•
5' - TACGCGGT•
```

One-mer difference

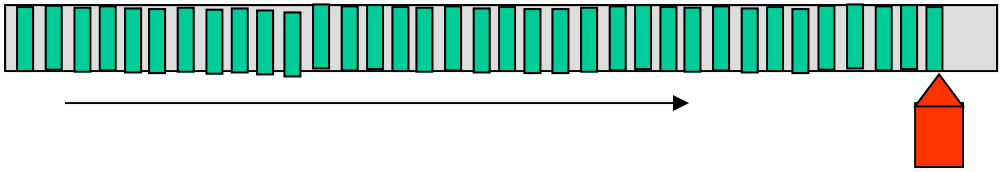


Electrophoresis

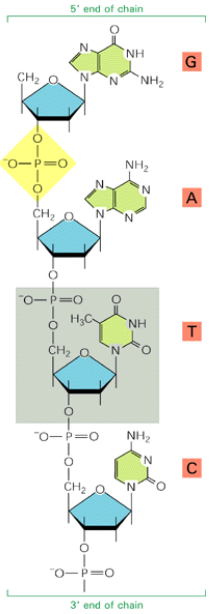


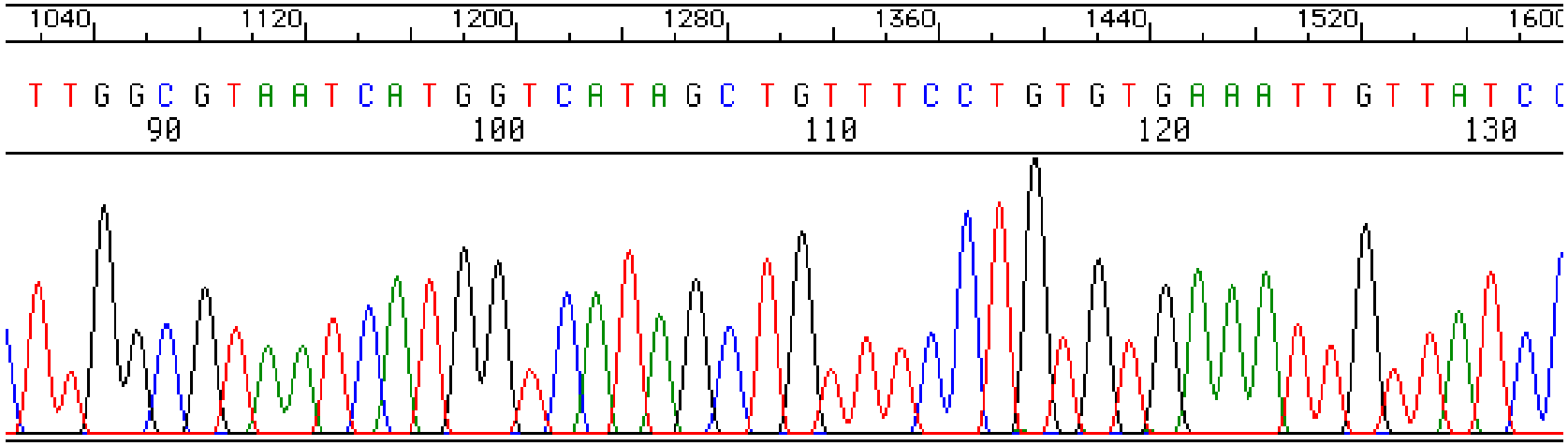
Automatic DNA Sequencing

One –lane: capillary electrophoresis



G, T, G, and C terminators are labeled by fore different dyes respectively.





A.Important Concepts:

Electrophoresis

Electrophoretic mobility → **Separation**

Capillary electrophoresis

Electroosmosis and Electroosmotic flow → **Driving force**

Apparent Mobility

Separation Efficiency

B. Modes of separation capillary electrophoresis

1. Capillary Zone electrophoresis
2. Capillary iso-electric focusing
3. Micellar electrokinetic Capillary chromatography
4. Capillary electrochromatography
5. Capillary gel electrophoresis

C. Bio-Applications

Separation Sciences

1. Introduction: Fundamentals of Distribution Equilibrium

2. Gas Chromatography (Chapter 2 & 3)

3. Liquid Chromatography (Chapter 4 & 5)

4. Other Analytical Separations (Chapter 6-8)
    a. Planar chromatography
    b. Supercritical fluid chromatography
    c. Electrophoresis
    d. Centrifugation
    e. Field Flow Fractionation

Homework III (b)

1. What is electrophoresis and electroosmosis?

2. Explain how neutral molecules can be separated by micellar Electrokinetic capillary chromatography.

3. Compare HPLC and Capillary electrochromatography.

4. Compare capillary gel electrophoresis and gel electrophoresis.

5. The observed behavior of benzyl alcohol ($C_6H_5CH_2OH$) in capillary electrophoresis is given below. Explain what happens as voltage is increased.

Electric field (V/m)	Number of plates
6400	38000
12700	78000
19000	96000
25500	124000
31700	124000
38000	96000