# Química Analítica Instrumental I


F.Q. UNAM



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# Why Does an Ion Subjected to a Constant Force Move at Constant Velocity?

A particle with charge, $Q$, when subjected to an electric field (voltage gradient, electrical "pressure" gradient), $E$, experiences a constant net force

$$m\,a = QE \qquad (7\text{-}1)$$

$m$ = mass

$a$ = acceleration

$$\boxed{m\,a = QE - f\frac{dy}{dt}} \qquad (7\text{-}2)$$

$$QE = f\frac{dy}{dt}$$

or

$$\boxed{\text{limiting velocity} = \frac{dy}{dt} = \frac{QE}{f}} \qquad (7\text{-}3)$$

$$\text{average translational kinetic energy} = \frac{1}{2}\,m\,v_{\text{thermal}}^2 = \frac{3}{2}\,kT \qquad (7\text{-}4)$$

$\tau$ = average time between collisions (sec/collision)
$\nu$ = frequency of collisions (collisions/sec)
$l$ = average distance between collisions (distance/collision)
$v_{\text{th}}$ = thermal velocity (distance/sec)

$$\tau = \frac{1}{\nu} \qquad (7\text{-}5)$$

$$m\,a = QE \tag{7-1}$$

or

$$a = \frac{QE}{m} \tag{7-6}$$

$$\text{Average net forward velocity between collisions} = \frac{QE\,\tau^2}{2m\,\tau} = \frac{QE\tau}{2m} \tag{7-7}$$

$$\mu = \frac{QE\tau}{2mE} = \frac{Q\tau}{2m} \tag{7-8}$$

$$\nu\tau = 1 \tag{7-9}$$

and substituting for $\tau$,

$$\tau = (l/v_{\text{th}}) \tag{7-10}$$

one obtains

$$\mu = \frac{Q\,\nu\,l^2}{2m\,v_{\text{th}}^2} \tag{7-11}$$

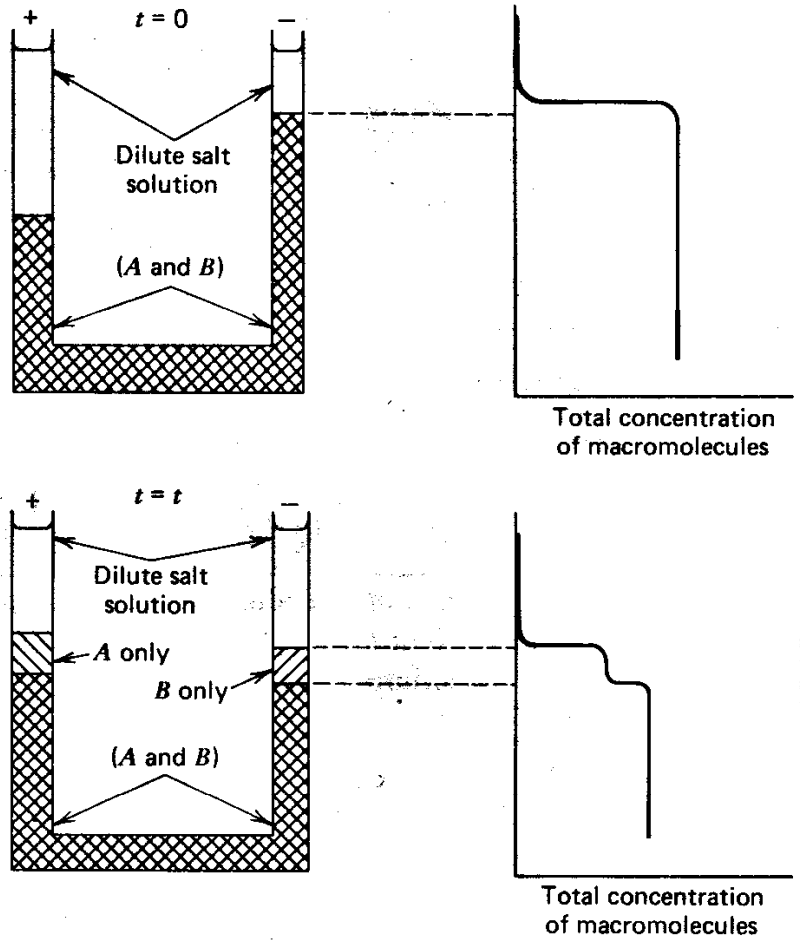



**FIGURE 7-1.** Separation of two macromolecules, $A$ and $B$, in an early electrophoresis apparatus. Both molecules are negatively charged, and $A$ moves faster than $B$ under influence of an electric field. Total macromolecule concentration is plotted as a function of distance along the descending "limb" of the device.

$$\text{Diffusional flow} = -D\frac{\partial[C]}{\partial y} \qquad (6\text{-}51)$$

$$\text{Electrophoretic flow} = (\mu E)[C] \qquad (7\text{-}14)$$

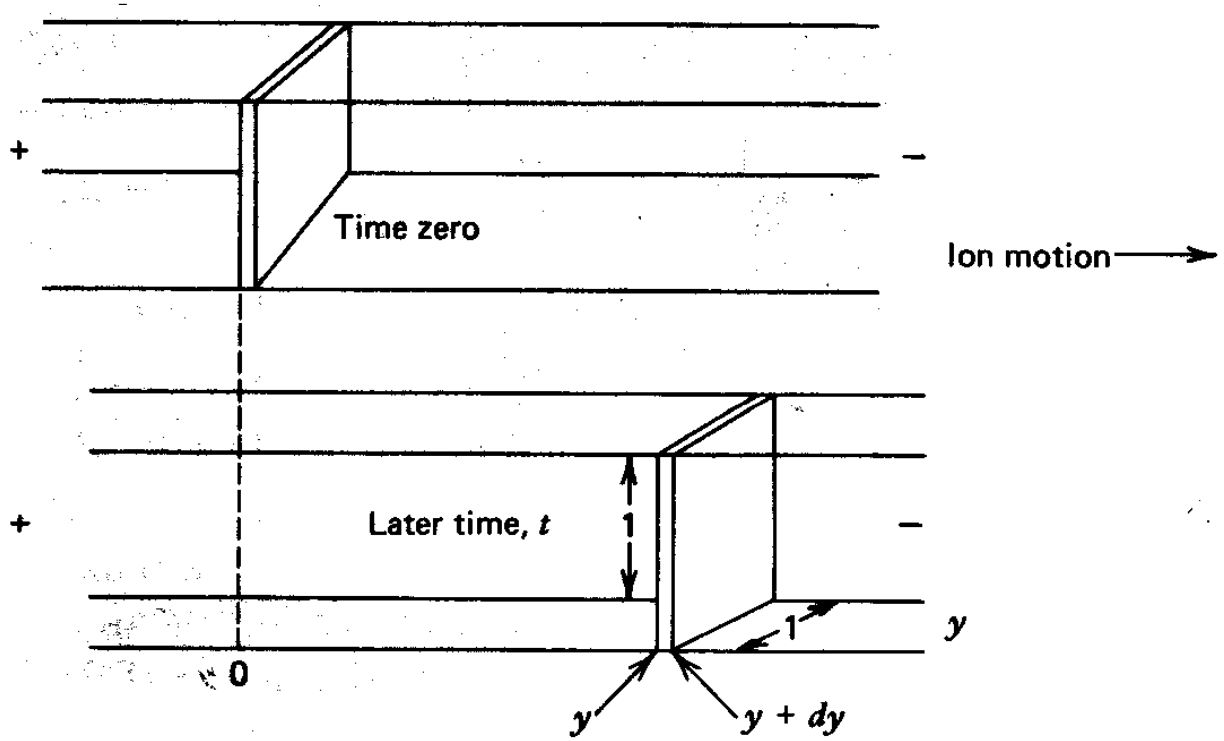



**FIGURE 7-2.** Diagram for analysis of gel electrophoresis experiments. Substance begins in a narrow band at $y = 0$ at $t = 0$; at later $t = t$, the flow of substance in and out of the region of unit cross-sectional area bounded by $y$, $y + dy$, is analyzed. The macro-ion is taken to be positively charged, so that it will move to the right when the electric field is turned on.

$$\frac{\partial[C]}{\partial t} = \frac{-D(\partial[C]/\partial y)_{\text{at } y} + \mu E[C]_{\text{at } y} + D(\partial[C]/\partial y)_{\text{at } y+dy} - \mu E[C]_{\text{at } y+dy}}{1 \cdot 1 \cdot dy} \tag{7-15}$$

$$[C]_{\text{at } y+dy} \cong [C]_{\text{at } y} + \left[\frac{\partial[C]}{\partial y}\right]_{\text{at } y} dy \tag{6-54}$$

$$\frac{\partial[C]}{\partial y}_{\text{at } y+dy} \cong \frac{\partial[C]}{\partial y}_{\text{at } y} + \left[\frac{\partial}{\partial y}\frac{\partial[C]}{\partial y}\right]_{\text{at } y} dy \tag{7-16}$$

$$\frac{\partial[C]}{\partial t} = D\frac{\partial^2[C]}{\partial y^2} - \mu E\frac{\partial[C]}{\partial y} \tag{7-17}$$

$$y' = y - \mu E t \tag{7-18}$$

$$\frac{\partial[C]}{\partial t} = D\frac{\partial^2[C]}{\partial (y')^2} \tag{7-19}$$

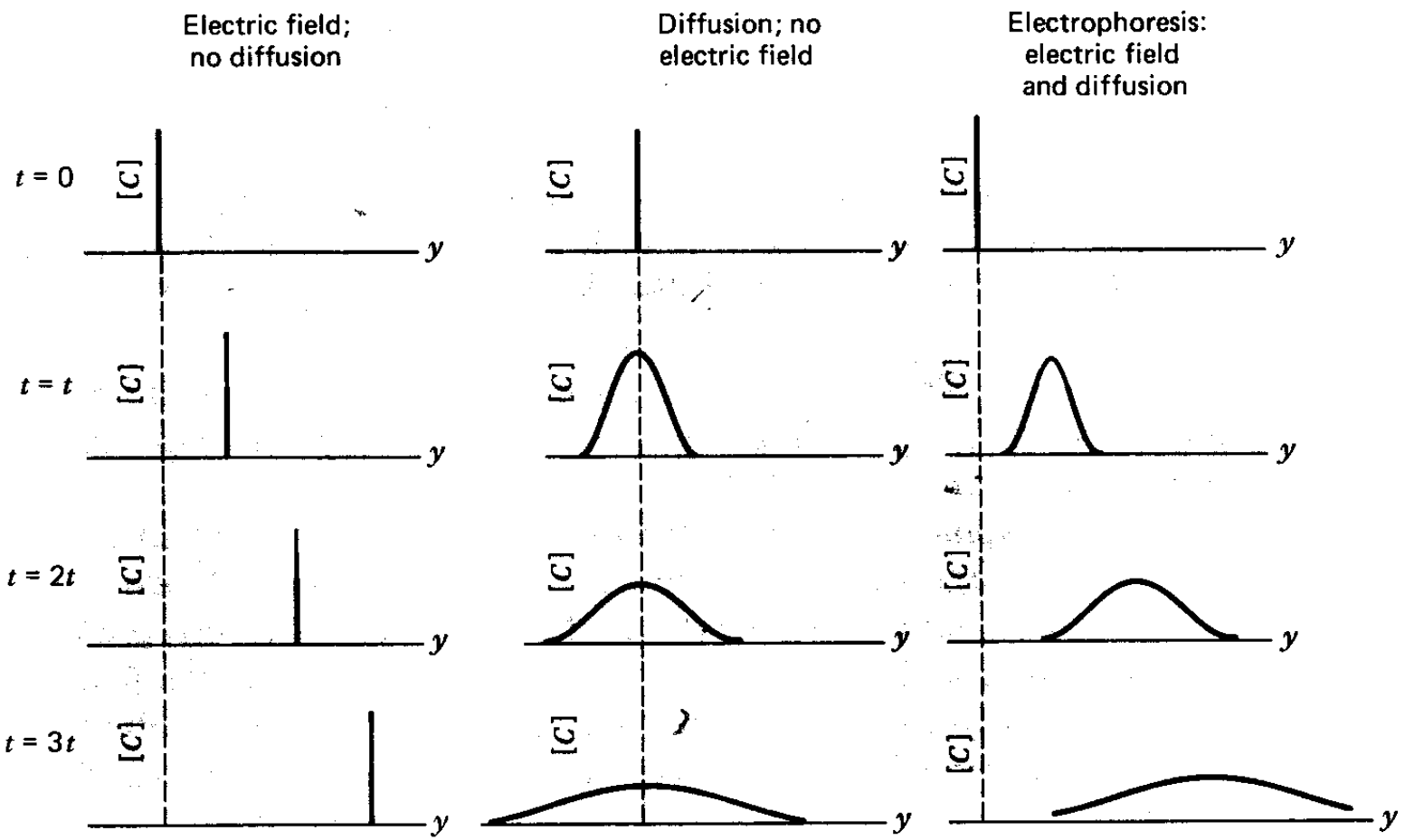



**FIGURE 7-3.** Plots of ion concentration versus distance under various conditions. Effect of electric field alone is given by Eq. 7-3; effect of diffusion alone is given by Equations 6-55 and 6-56; effect of diffusion plus electric field (electrophoresis) is given by Equations 7-17 and 7-19.


**Figure 6-6.** Commercially available chambers for acylamide gel electrophoresis of short and long gels. Arrows in the left apparatus point to the platinum electrode wires. (Courtesy of Savant Instruments, Inc., Hicksville, N.Y.)

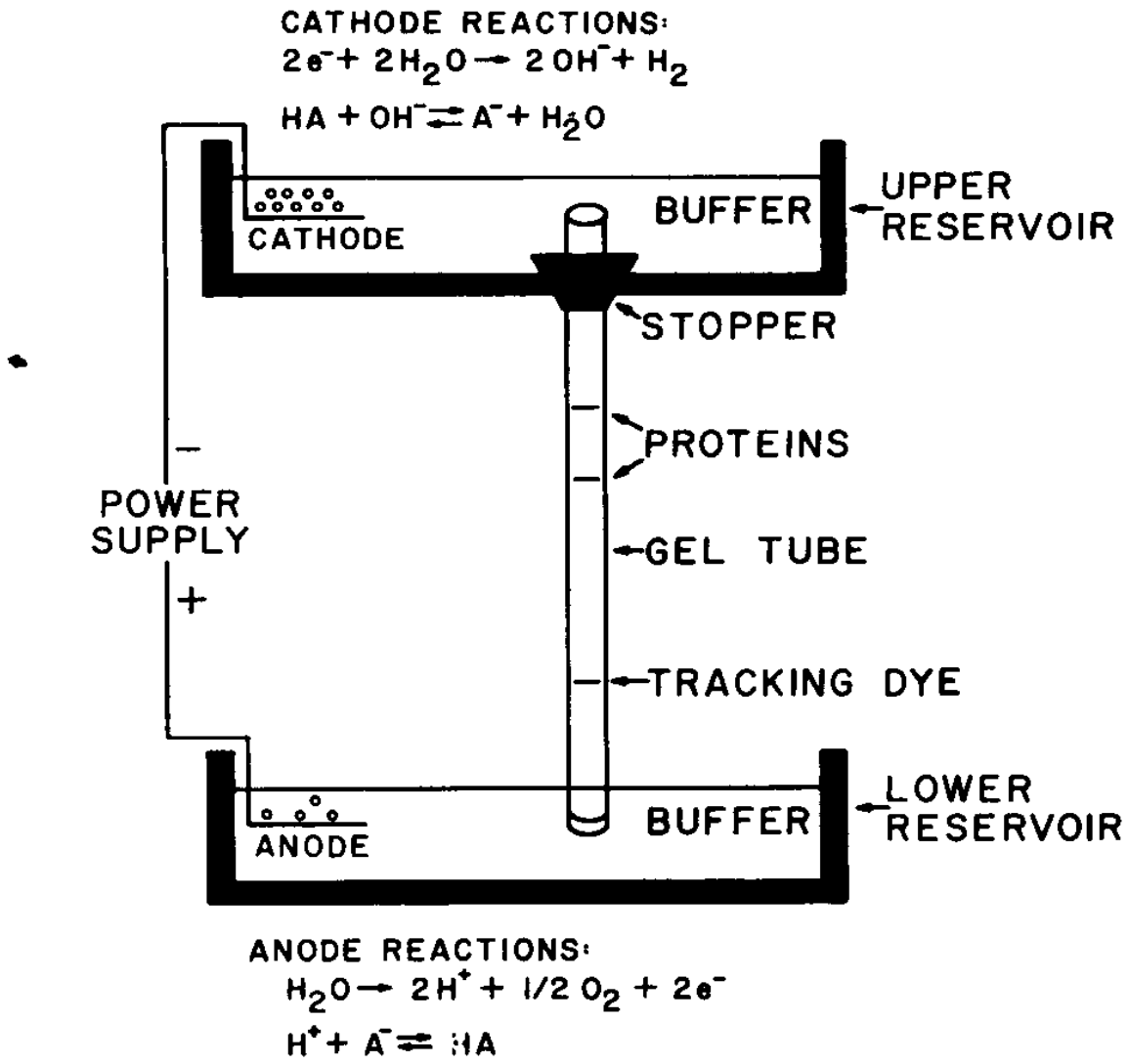



**Figure 6-7.** Diagram of an electrophoresis apparatus showing the reactions that occur at each electrode.

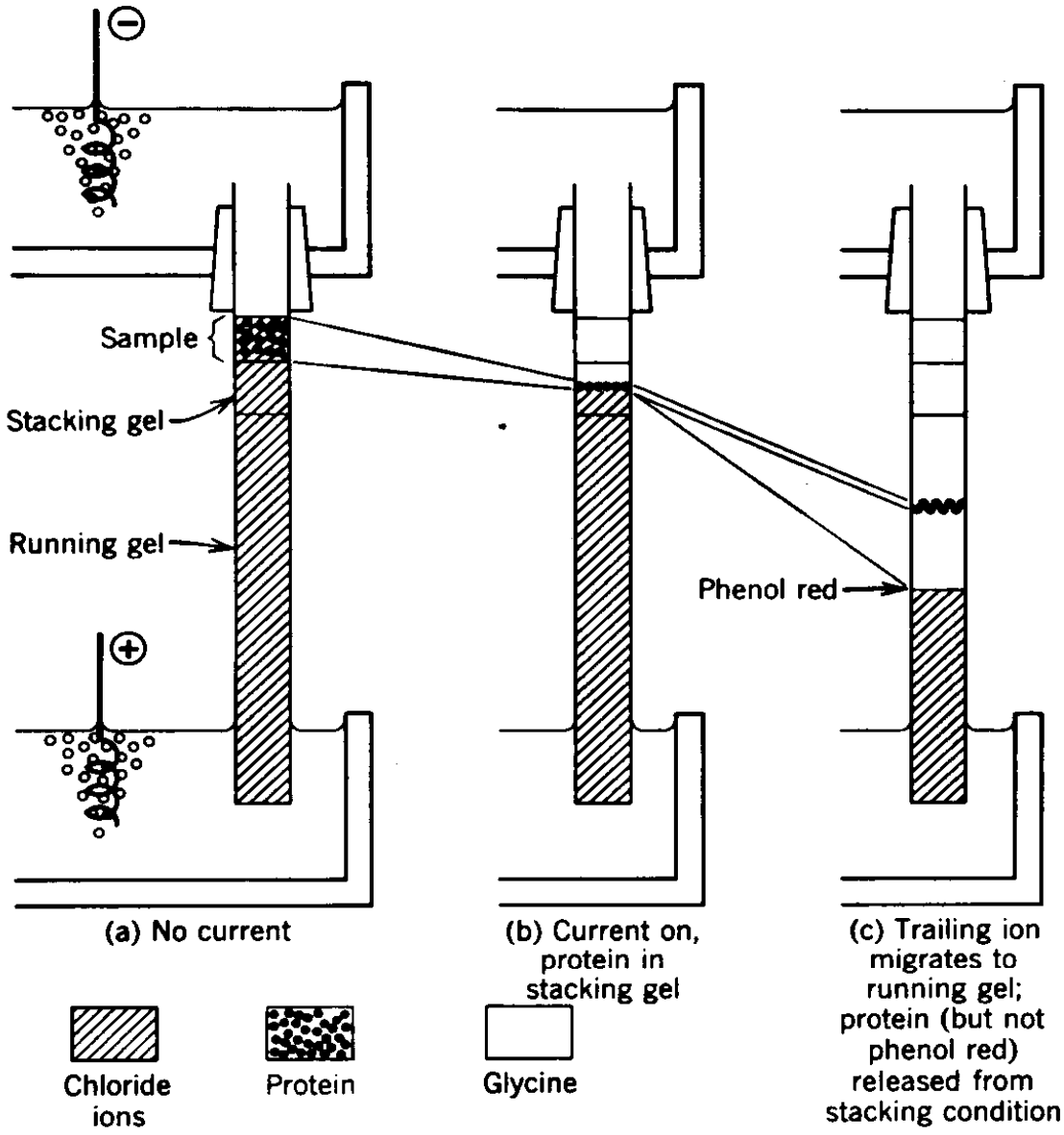



**Figure 6-9.** Schematic diagram of a two gel system and the movement of the various ionic species during electrophoresis. (From G. Bruening, R. Criddle, J. Preiss, and F. Rudert, *Biochemical Experiments*, Wiley, New York, 1970.)

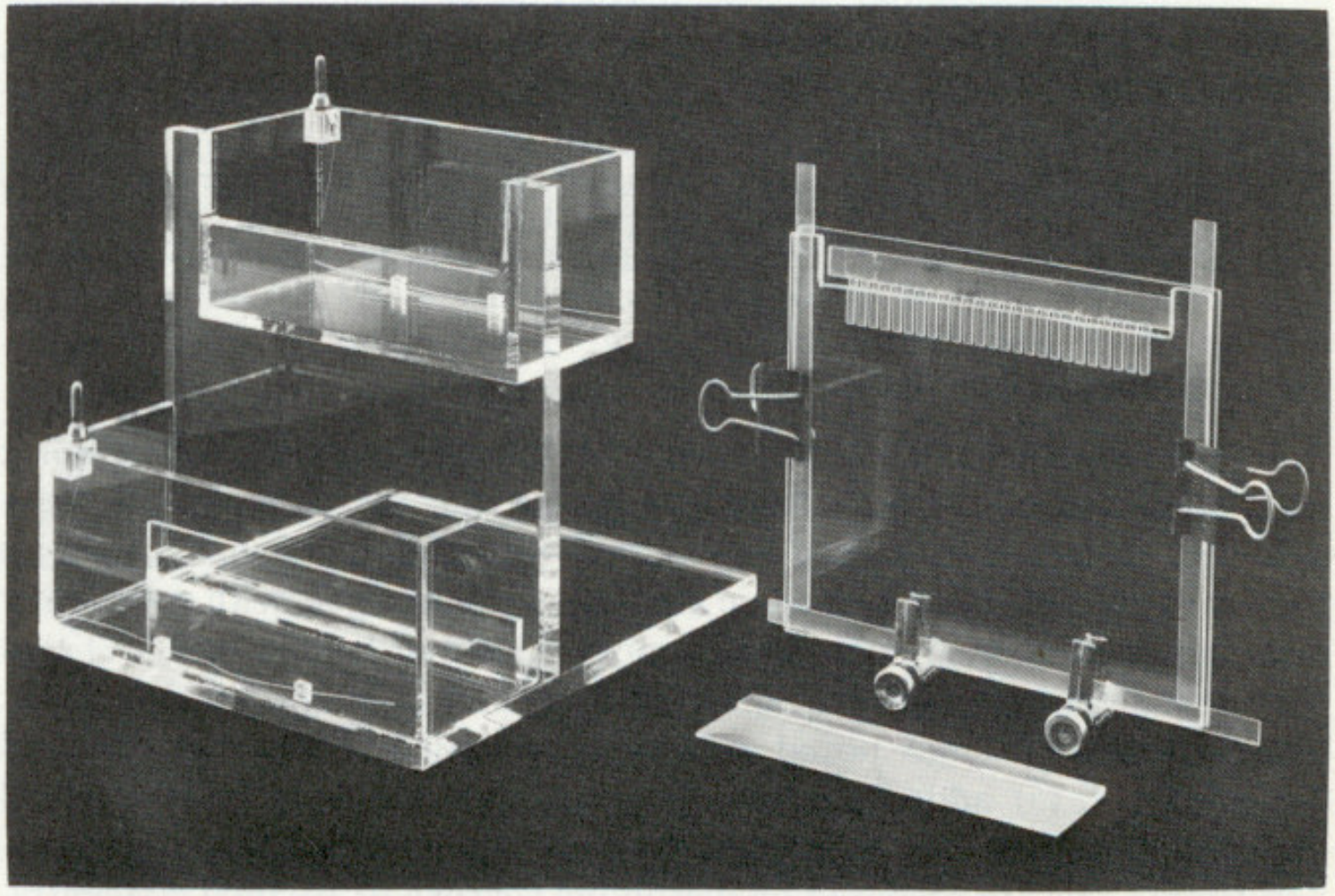


**Figure 6-12.** Slab gel apparatus. The glass plates are shown assembled with the side and bottom spacers in place and the comb inserted at the notch. [From F. W. Studier, *J. Mol. Biol.*, **79**:237 (1973).]


**FIGURE 7-4.** Electrophoretic pattern of normal human serum proteins for agar gel electrophoresis: anode at right, cathode at left. (After L. P. Cawley, *Electrophoresis and Immunoelectrophoresis,* Little, Brown & Co., Boston, 1969, p. 12.)

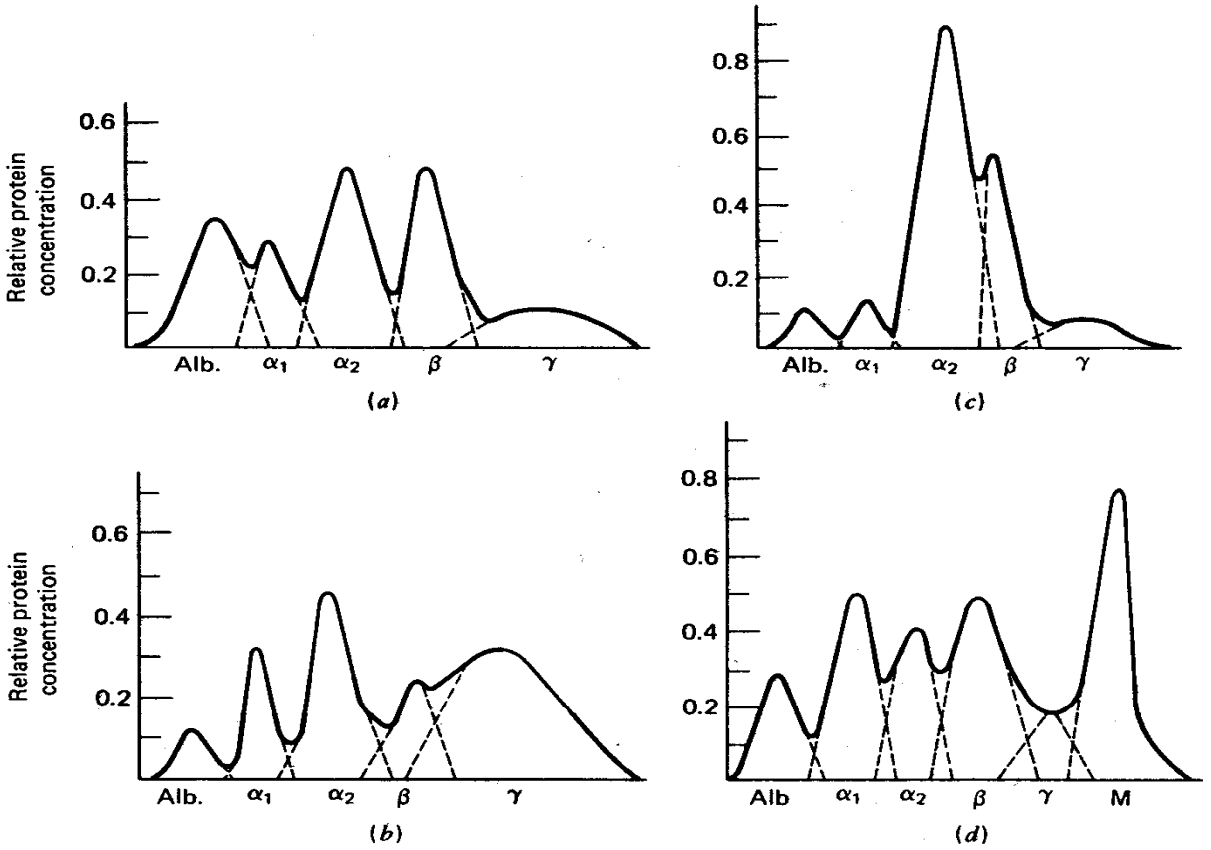
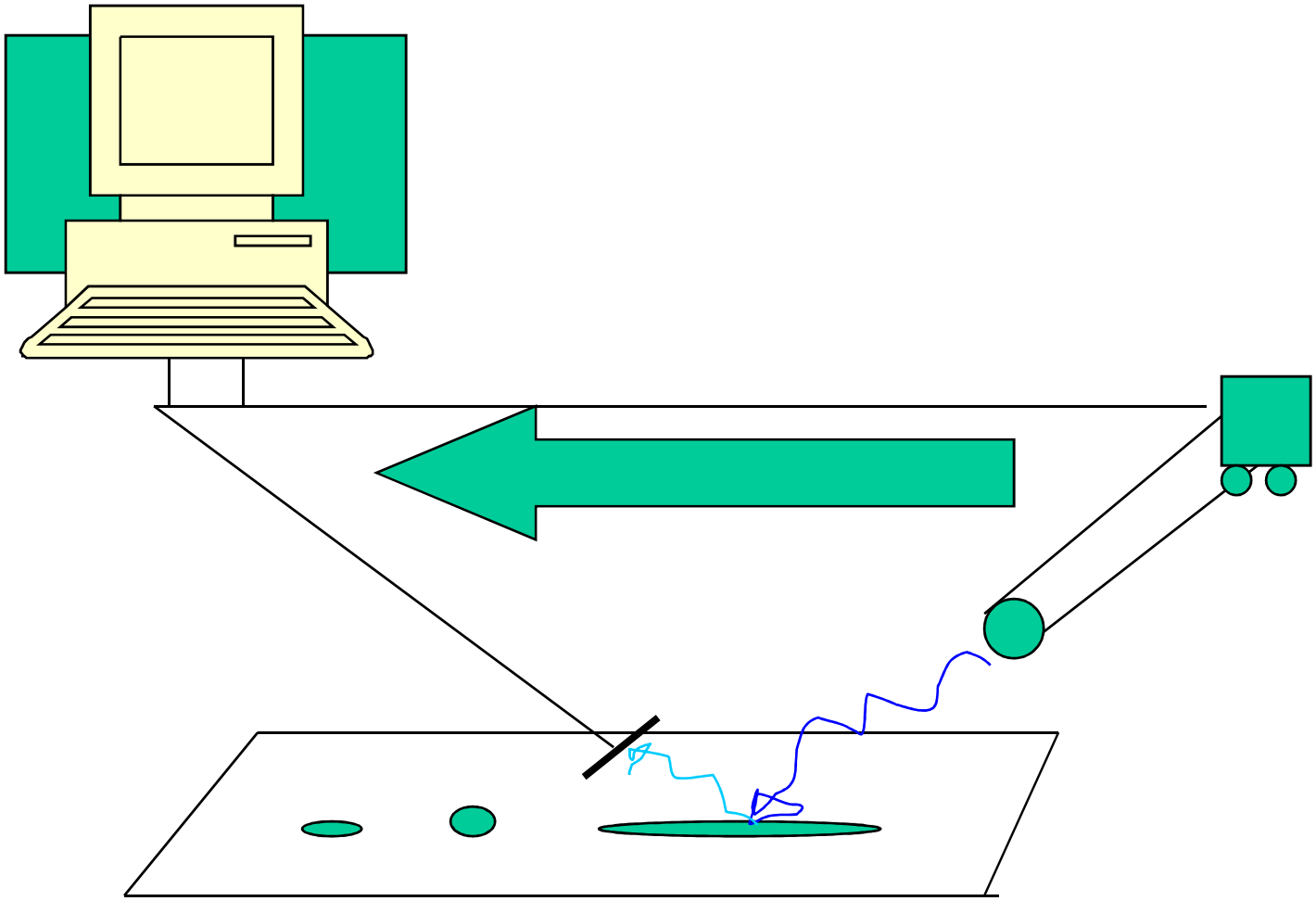
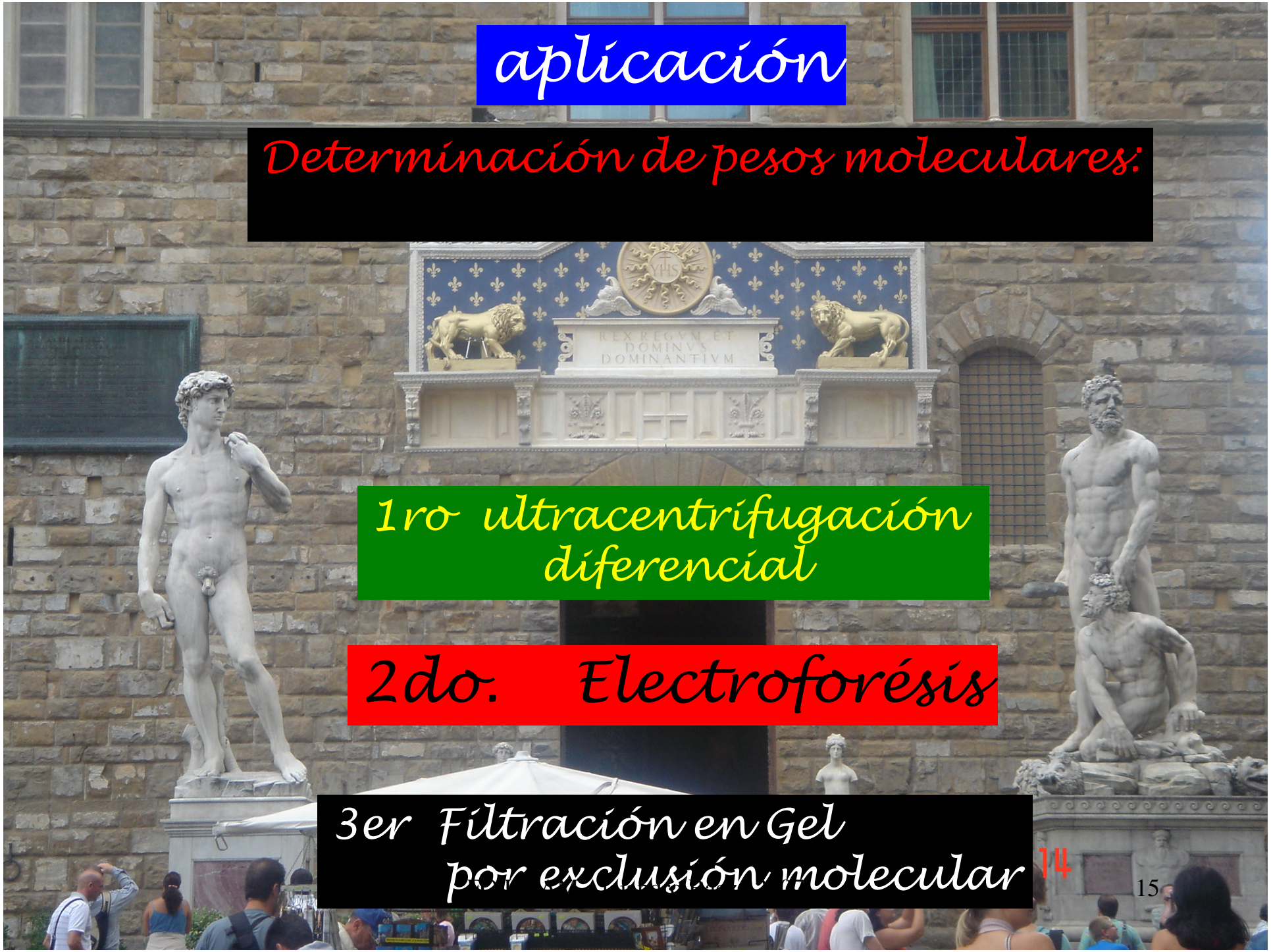



**FIGURE 7-5.** Cellulose acetate gel electrophoresis patterns for (*a*) normal serum, (*b*) infectious hepatitis, (*c*) lipid nephrosis, and (*d*) gamma myeloma. Protein fractions are listed in Table 7-1. Protein concentration is determined from optical absorption (see Section 4). [From *Clinica Chimica Acta,* 672 (1960).]






# aplicación

## Determinación de pesos moleculares:

1ro ultracentrifugación diferencial

2do. Electroforésis

3er Filtración en Gel por exclusión molecular

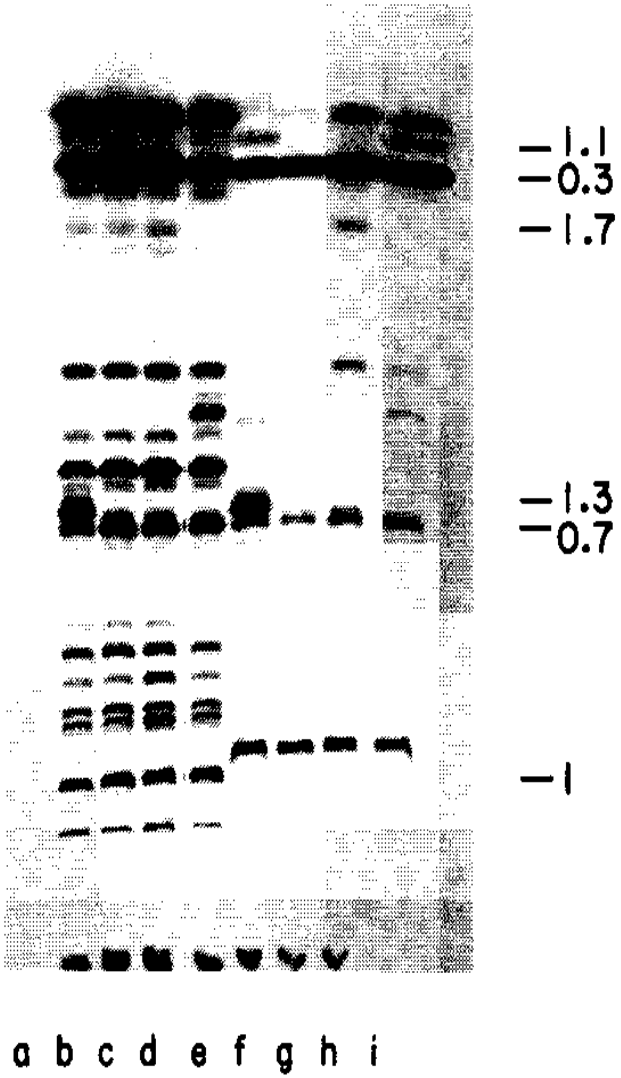



**Figure 6-13.** T7 proteins separated by SDS acrylamide slab gel electrophoresis. [From F. W. Studier, *J. Mol. Biol.*, **79**:237 (1973).]

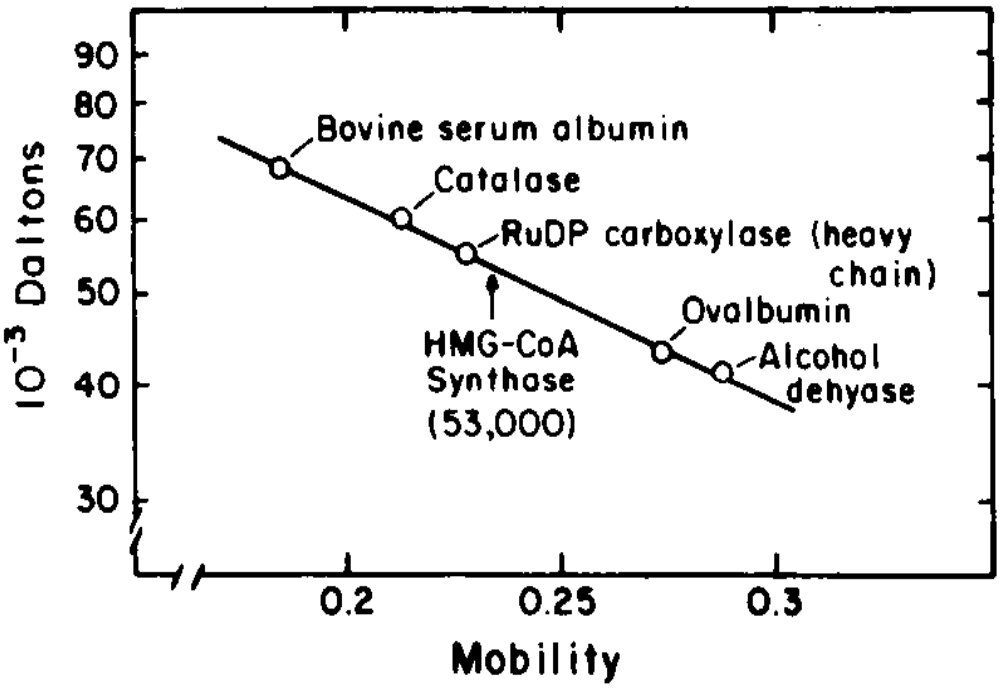



**Figure 6-11.** Estimation of the subunit mass of mitochondrial HMG–CoA synthase by SDS gel electrophoresis. [From W. D. Reed, K. D. Clinkenbeard, and M. D. Lane, *J. Biol. Chem.*, **250**:3120 (1975).]

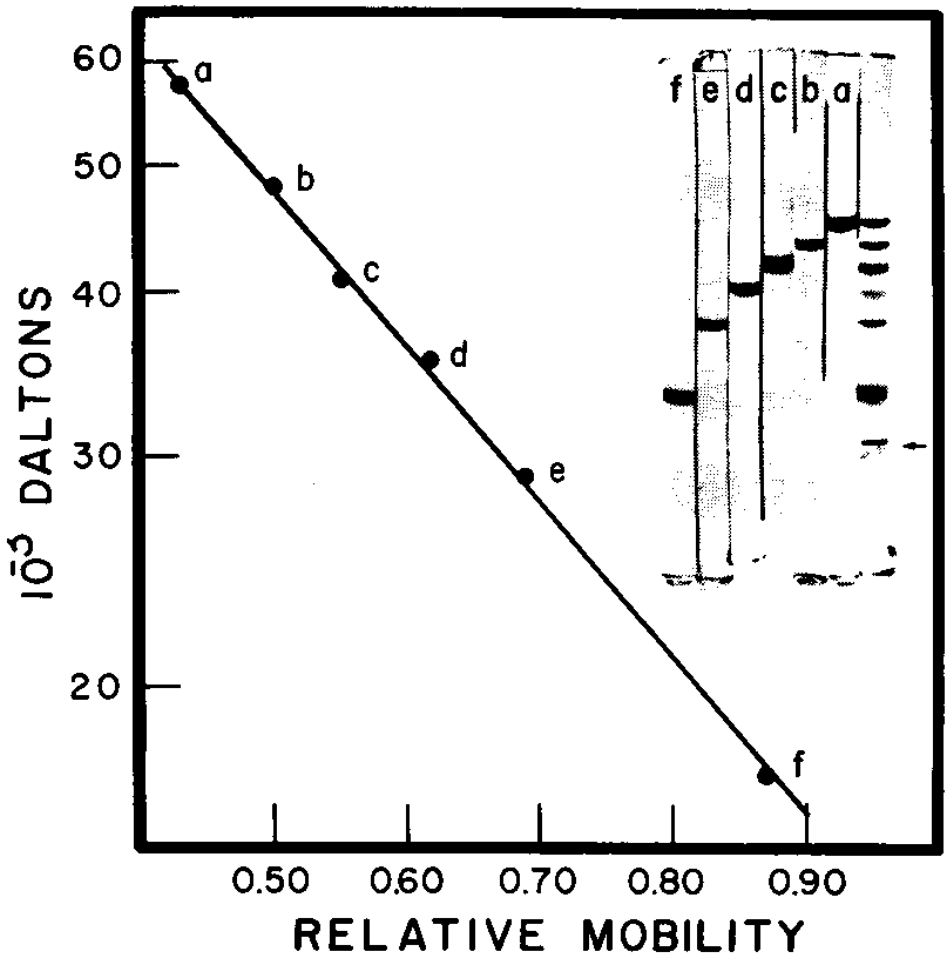
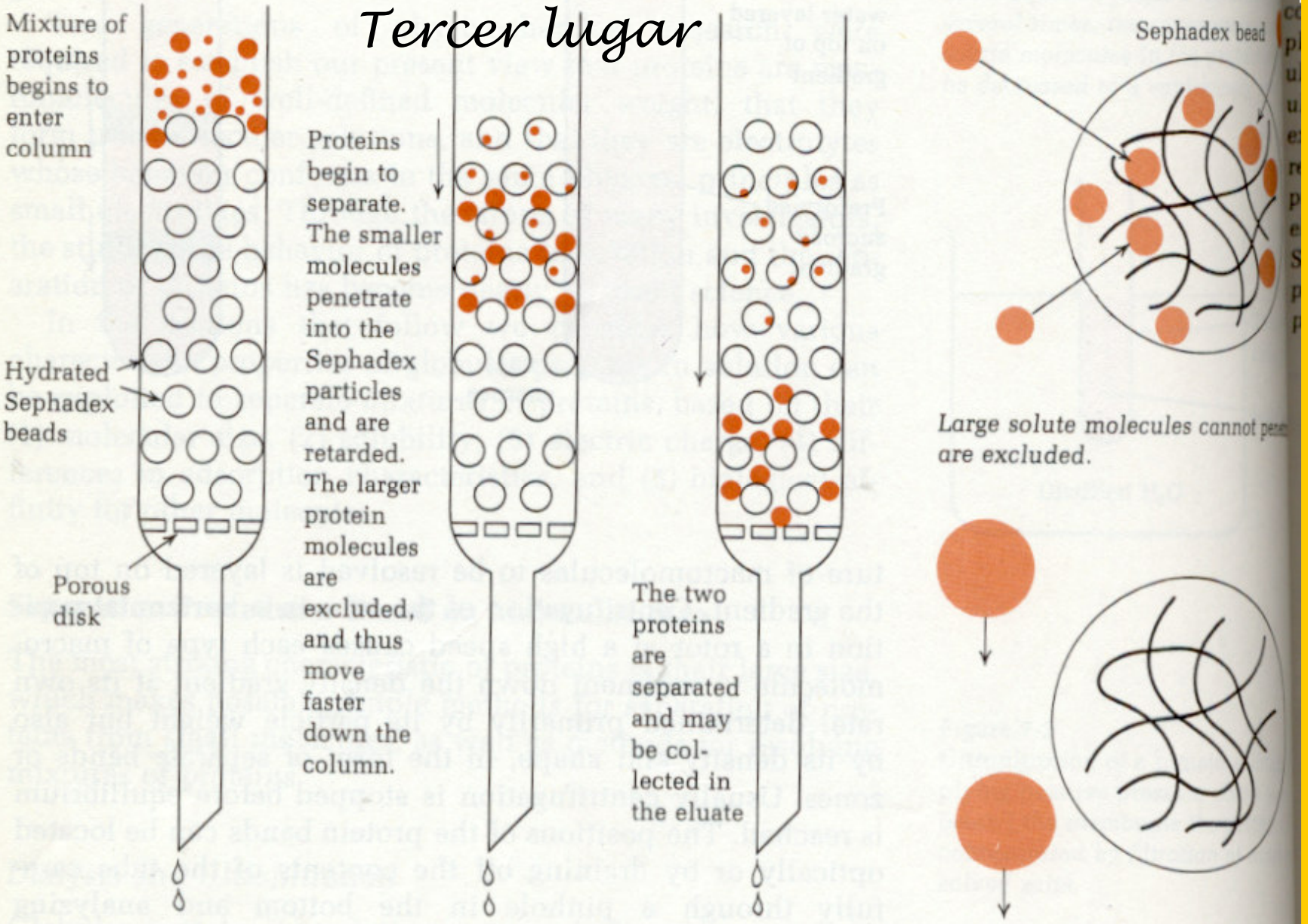



**Figure 6-26.** Separation of standard proteins using SDS acrylamide gel electrophoresis. The standards are *a*, catalase; *b*, fumarase; *c*, aldolase; *d*, glyceraldehyde-3P dehydrogenase; *e*, carbonic anhydrase; and *f*, myoglobin. The inset shows Coomassie Brilliant Blue staining of the acrylamide gels from which these data were derived. Arrow indicates the location of the dye marker. In this case it has been marked by inserting a small piece of nickel–chromium wire through the gel at the appropriate level.


Tercer lugar:
Exclusión molecular
Por filtración en Gel
20 13:48

# Tercer lugar



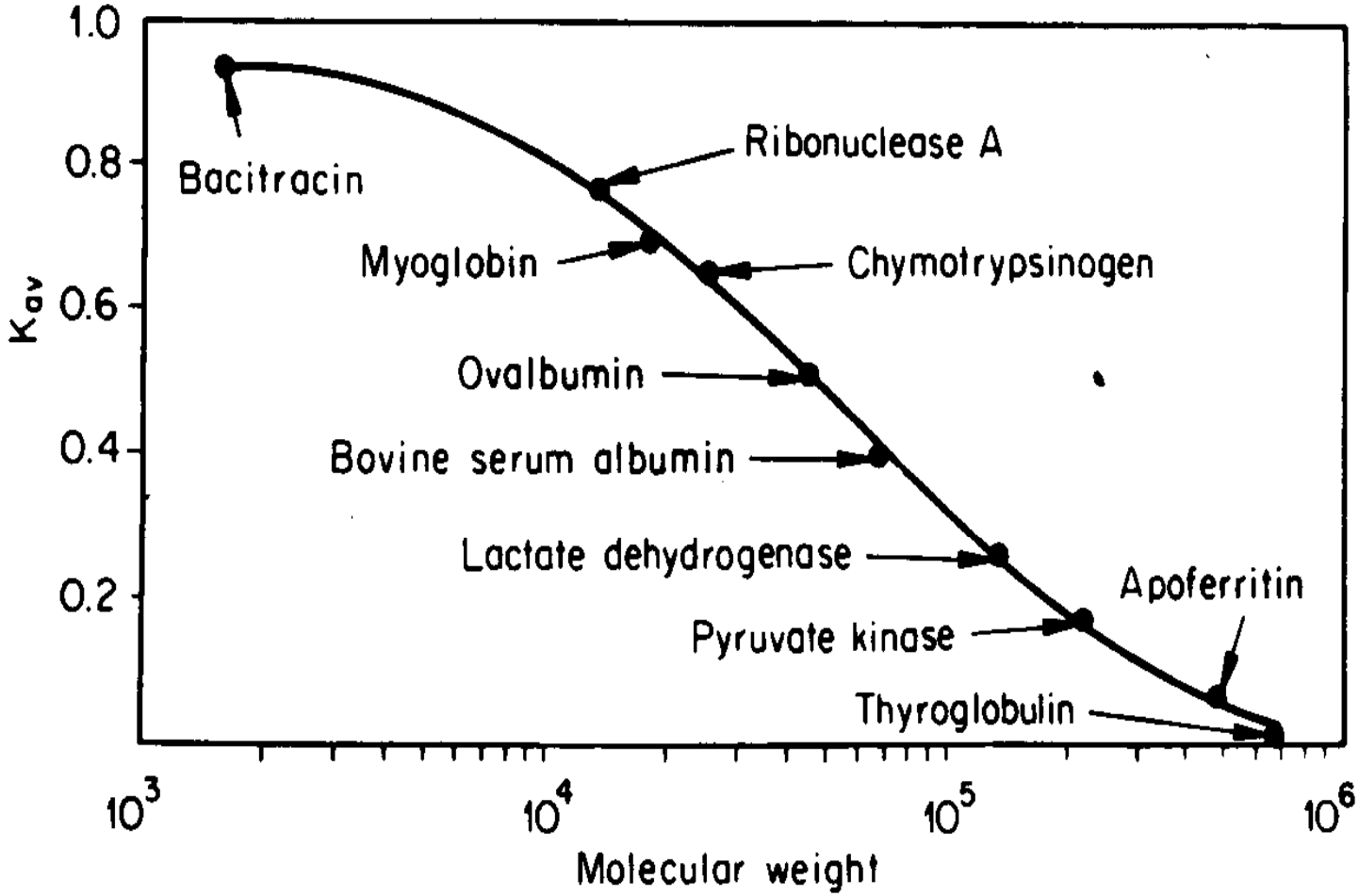



**Figure 5-3.** Selectivity curve for Sephadex G-200, showing $K_{av}$ values as a function of the molecular weights for several proteins. (Courtesy of I. M. Easterday, Pharmacia Fine Chemicals, Inc.)


# Primer lugar: Ultrtacentrifugación Diferencial.

# Primer lugar

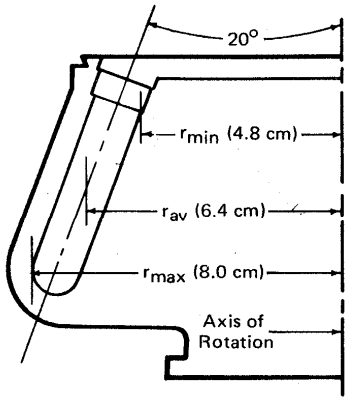



**Figure 9-1.** Cross-sectional diagram of an angle head rotor showing the distances from the axis of rotation to the top, middle, and bottom of the centrifuge tube. (Courtesy Spinco Division, Beckman Instruments, Inc., Palo Alto, Calif.)


**Figure 9-3.** Sorvall refrigerated highspeed centrifuge. Meters display the temperature and running speed; the two dials are used for selecting the speed and time of centrifugation. (Courtesy of DuPont Instruments, Newtown, Conn.)

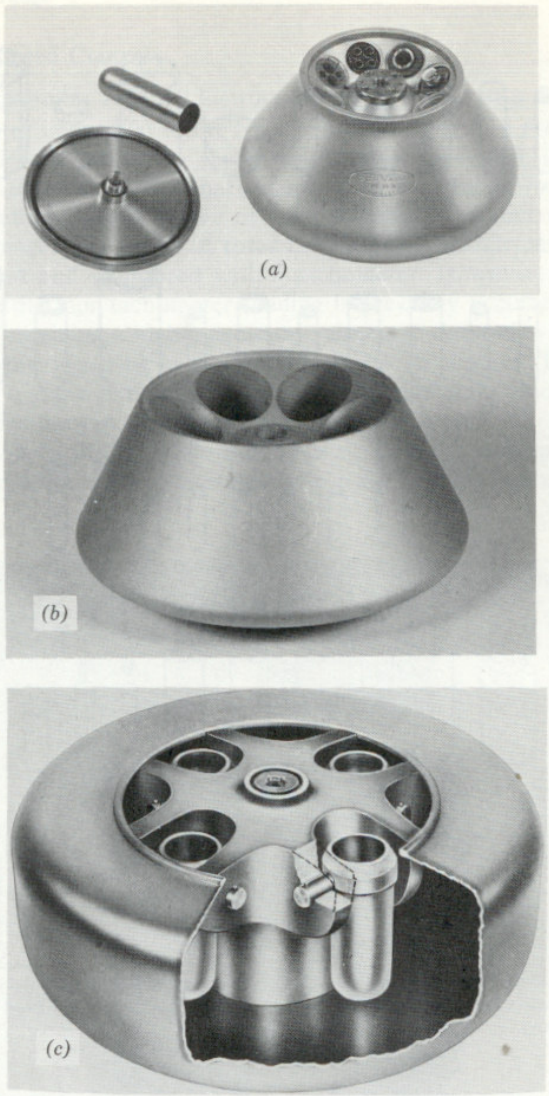



**Figure 9-4.** Various angle and swinging bucket rotors that may be used in a highspeed centrifuge. (*a*) The rotor accommodates eight 50 ml tubes or any of the adapters shown in the rotor. The concentric screws in the rotor cap are used to fasten the cap to the rotor (outer screw) and the rotor to the drive spindle (small inner screw). (*b*) The rotor accepts six 250 ml bottles. (*c*) The rotor is a swinging bucket model that accomodates four 50 ml tubes or appropriate adapters. The shield has been cut away to visualize the titanium yoke [illegible]. (Courtesy of DuPont Instruments, Newtown, Conn.)

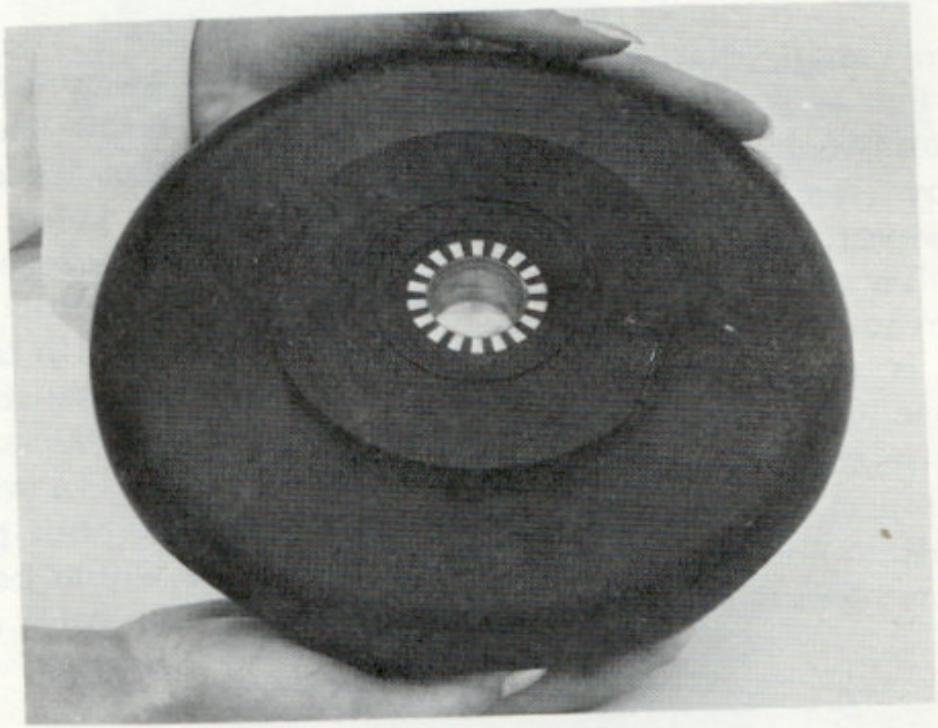


**Figure 9-7.** Rotor bottom showing the speed control ring consisting of alternating reflecting and nonreflecting surfaces. (Courtesy of Beckman Instruments, Palo Alto, Calif.)

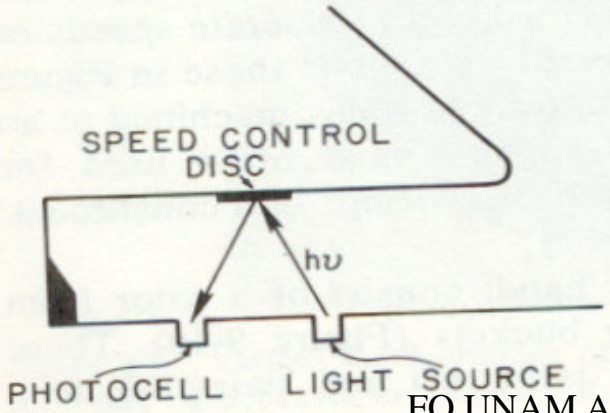
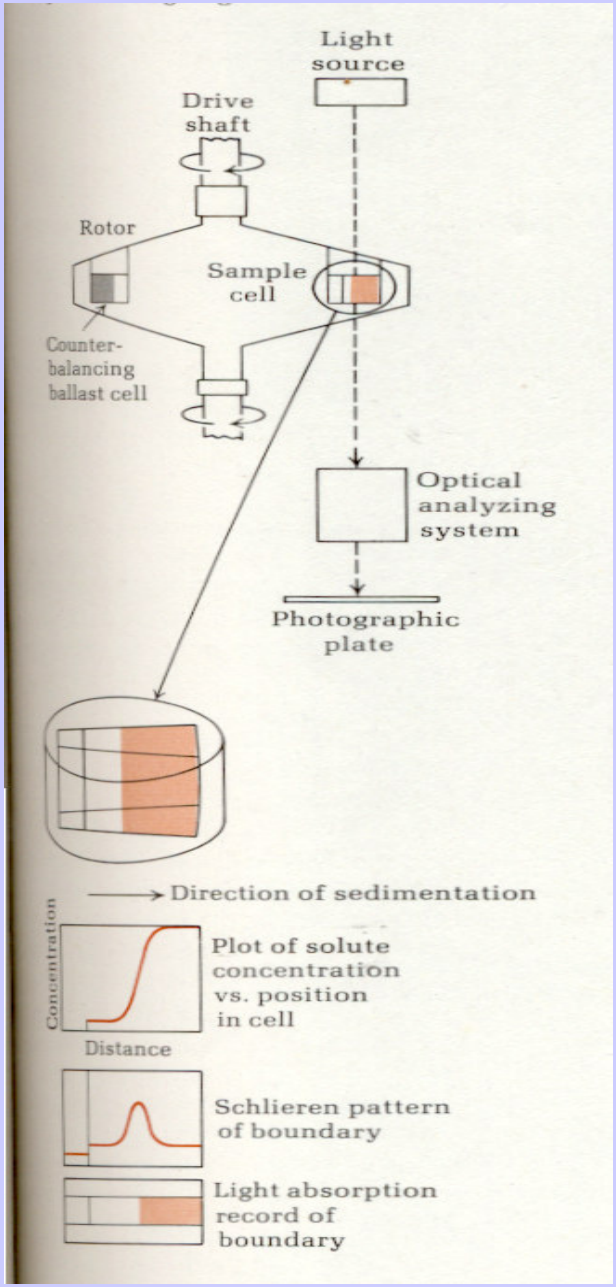
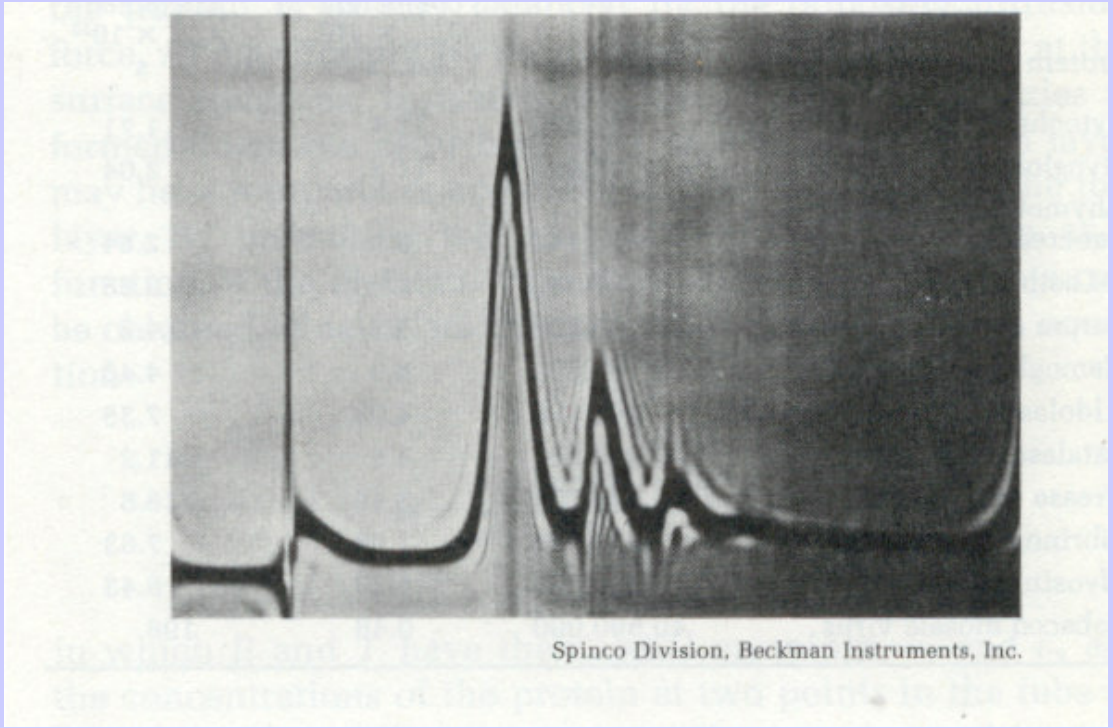



**Figure 9-8.** Diagram of the path followed by the light beam of an optical speed control system.



Spinco Division, Beckman Instruments, Inc.


El Renacimiento de la electroforésis
18 13:31


¡Electroforésis Capilar !
20 13:25

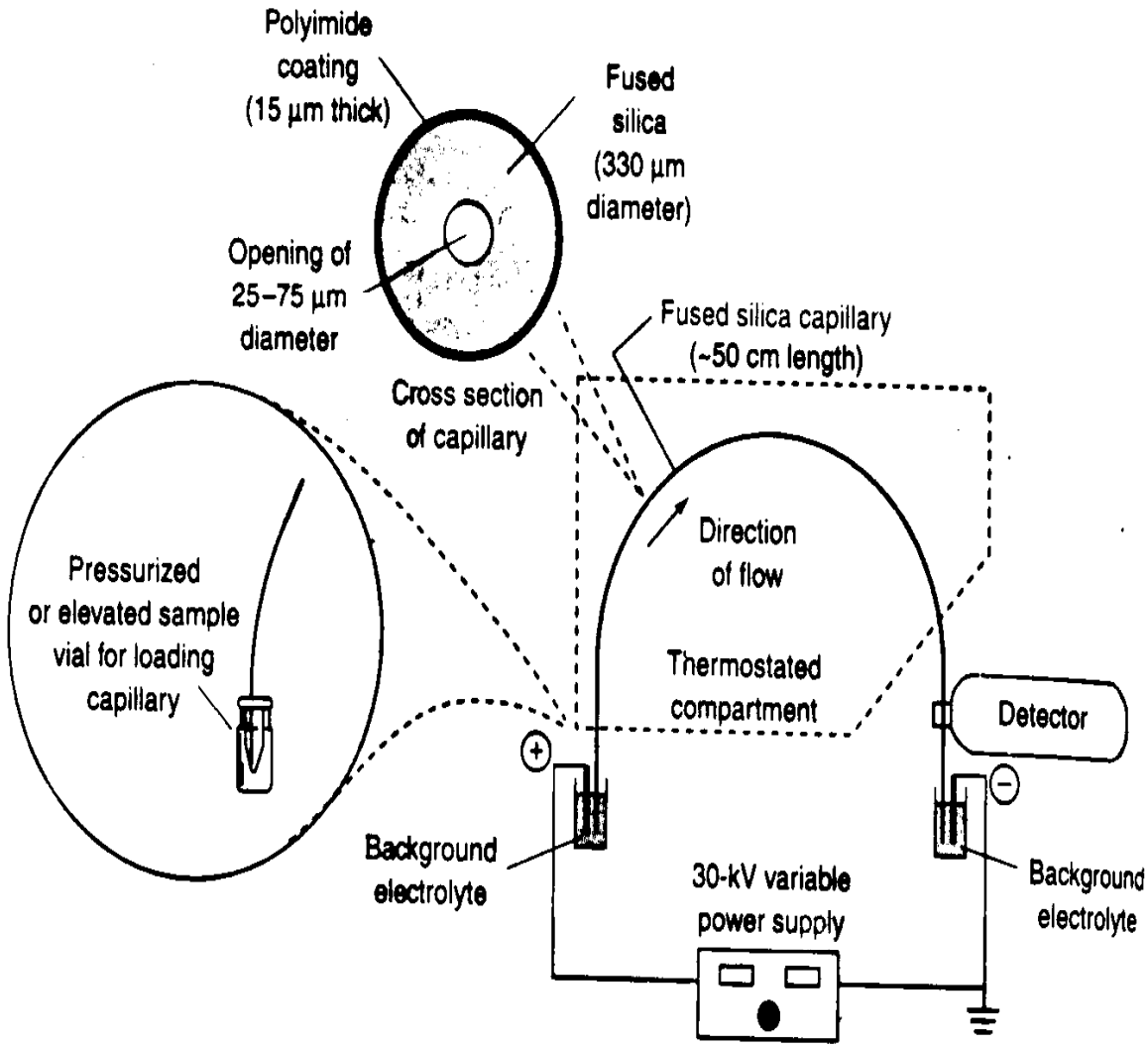
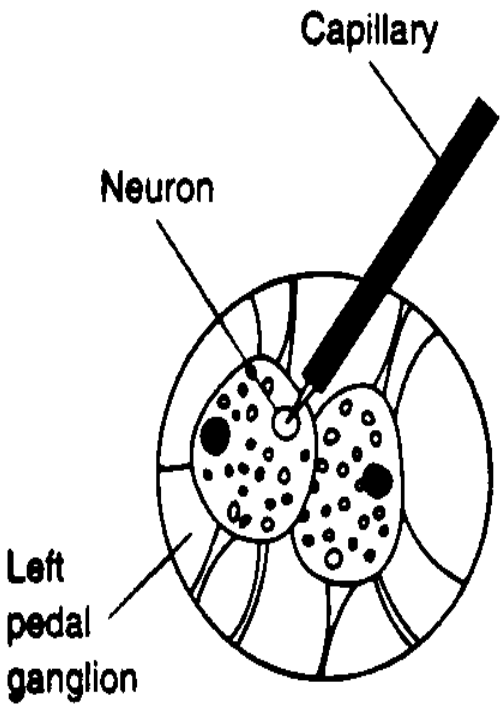
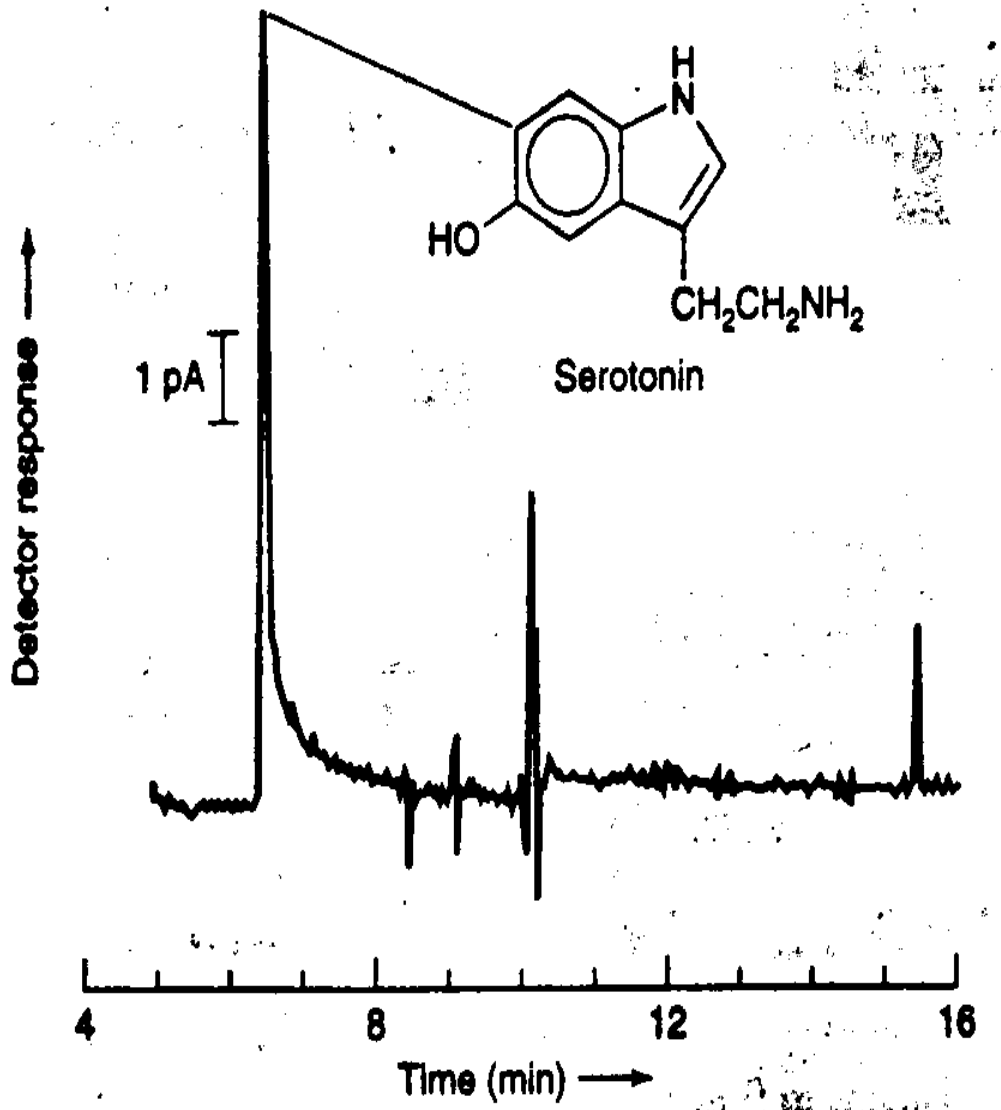



**Figure 26-13** Apparatus for capillary electrophoresis. One way to inject sample is to place the capillary in a sample vial and apply pressure to the vial or suction at the outlet of the capillary. The use of an electric field for sample injection is described in the text.


Capillary
Neuron
Left
pedal
ganglion


H
N
HO
CH2CH2NH2
Serotonin
1 pA
Detector response
4
8
12
16
Time (min)

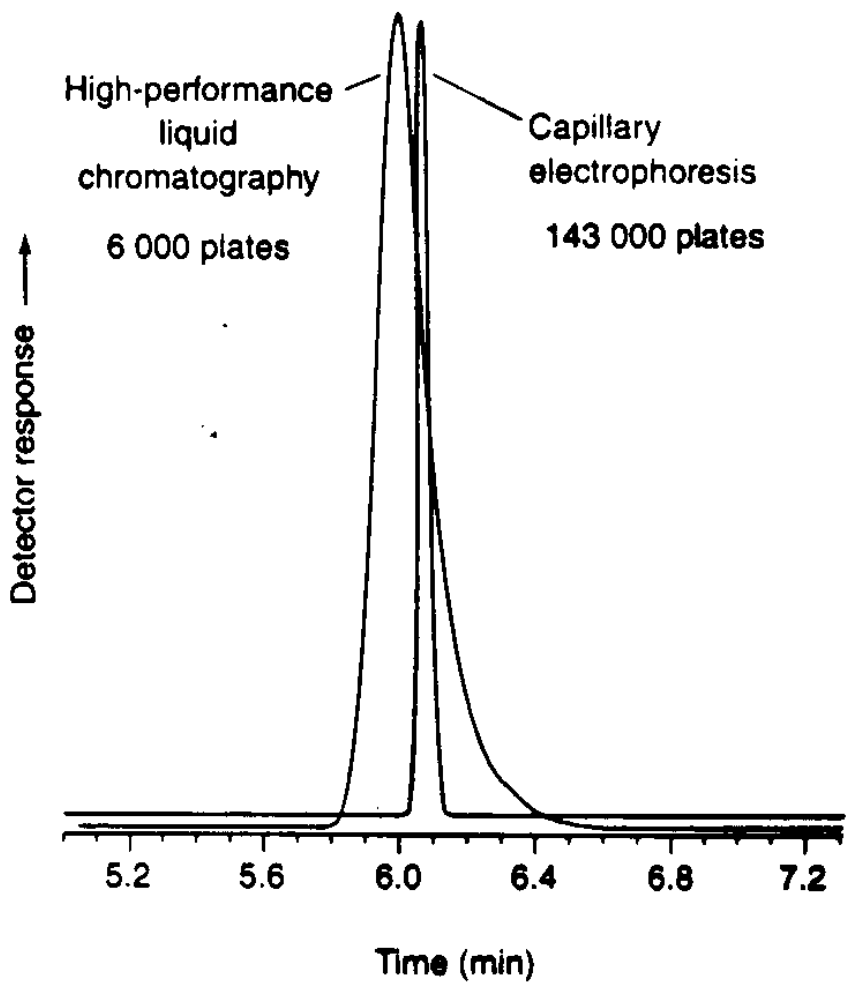



**Figure 26-14** Comparison of peak widths for benzyl alcohol ($C_6H_5CH_2OH$) in capillary electrophoresis and HPLC. [From S. Fazio, R. Vivilecchia, L. Lesueur, and J. Sheridan, *Am. Biotech. Lab.*, January 1990, p. 10.]

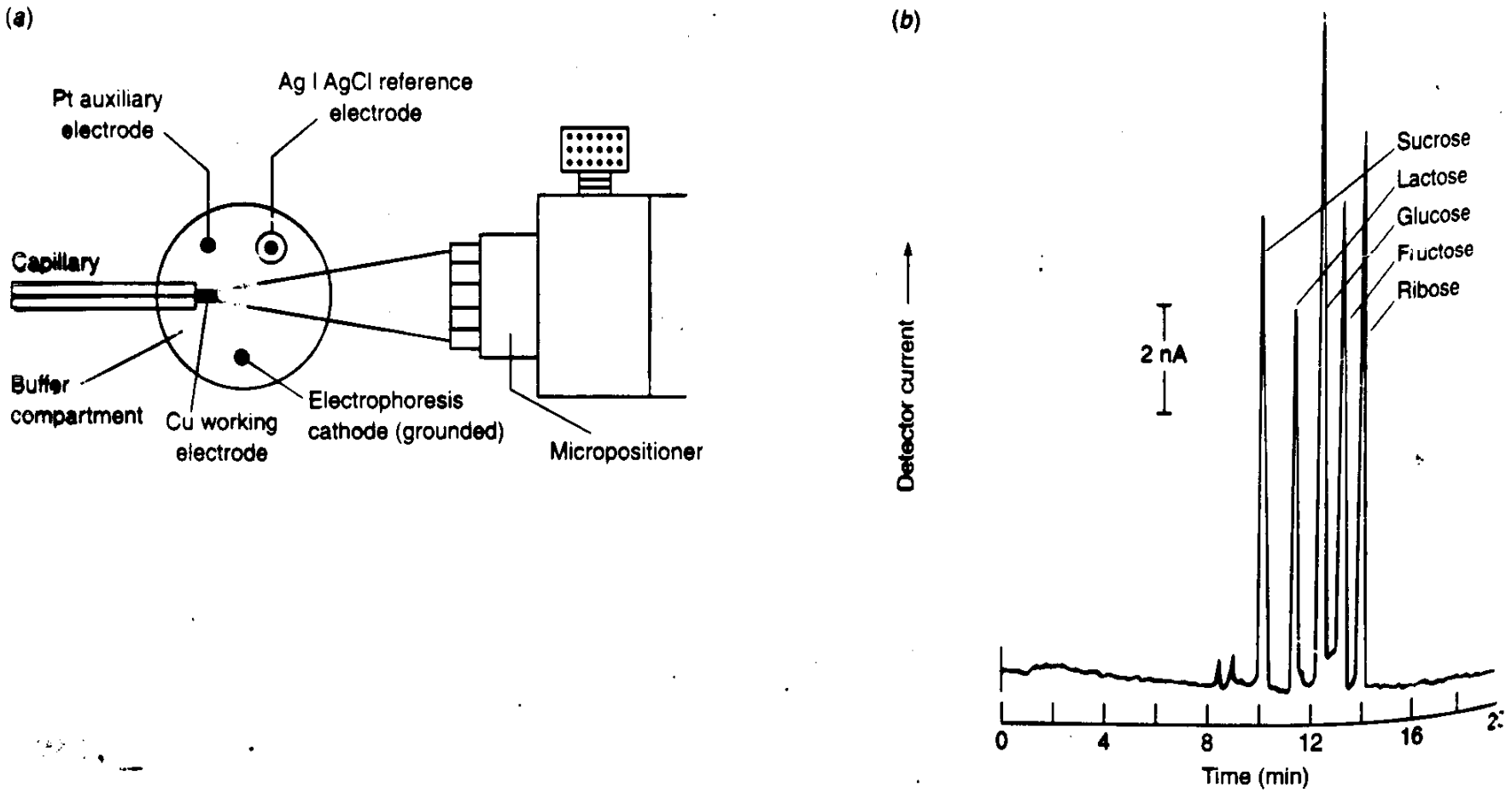



**Figure 26-22** (*a*) Amperometric detection with macroscopic working electrode at the outlet of the capillary. (*b*) Electropherogram of sugars separated in 0.1 M NaOH, in which OH groups are partially ionized, thereby turning the molecules into anions. [From J. Ye and R. P. Baldwin, *Anal. Chem.* **1993**, *65*, 3525.]

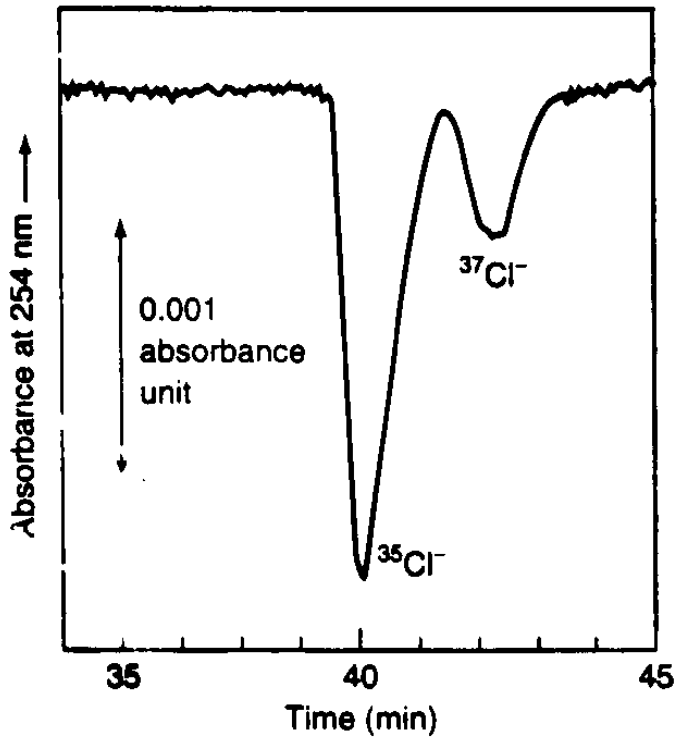
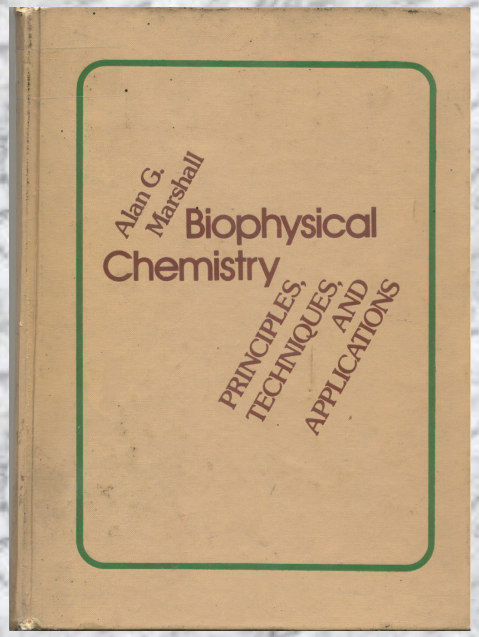



**Figure 26-24** Separation of natural isotopes of 0.56 mM $Cl^-$ by capillary electrophoresis with indirect spectrophotometric detection at 254 nm. The background electrolyte contains 5 mM $CrO_4^{2-}$ to provide absorbance at 254 nm, and 2 mM borate buffer, pH 9.2. The capillary had a diameter of 75 μm, a total length of 47 cm (length to detector = 40 cm), and an applied voltage of 20 kV. The difference in electrophoretic mobility of $^{35}Cl^-$ and $^{37}Cl^-$ is just 0.12%. Conditions were adjusted so that electroosmotic flow of solvent was nearly equal and opposite to the electrophoretic flow. The resulting near-zero net velocity gave the two isotopes maximum time to be separated by their slightly different rates of electrophoresis. [From C. A. Lucy and T. L. McDonald, *Anal. Chem.* **1995**, *67*, 1074.]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Biophysical Chemistry
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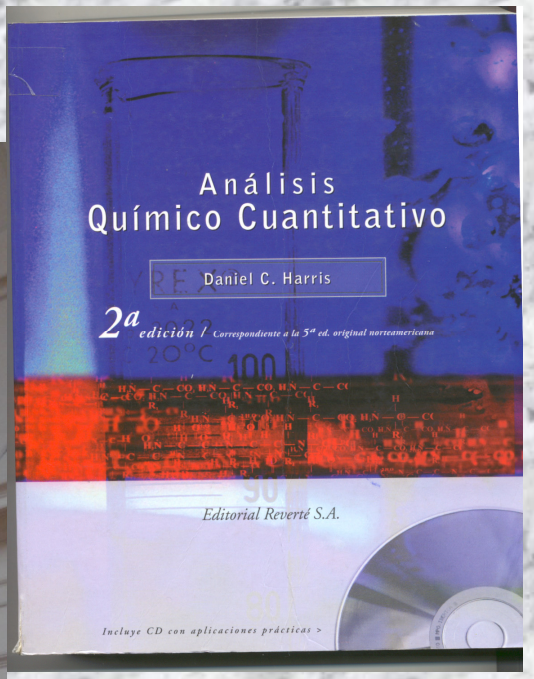
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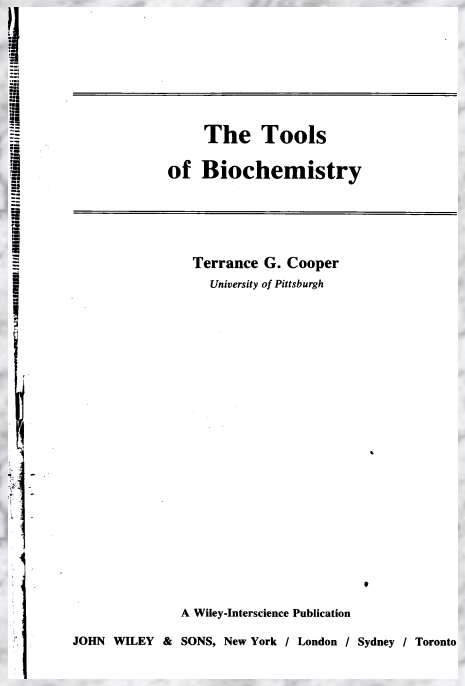
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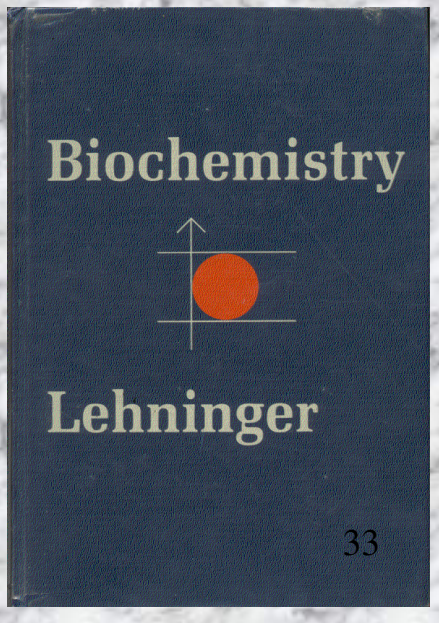
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