regard to any liquid remaining on the side of the flask, or the temperature observed when the proportion specified in the individual monograph has been collected.

NOTE—Cool all liquids that distill below 80° to between 10° and 15° before measuring the sample to be distilled.

Method I

Apparatus—Use apparatus similar to that specified for *Method II*, except that the distilling flask is of 50- to 60-mL capacity, and the neck of the flask is 10 to 12 cm long and 14 to 16 mm in internal diameter. The perforation in the upper insulating board, if one is used, should be such that when the flask is set into it, the portion of the flask below the upper surface of the insulating material has a capacity of 3 to 4 mL.

Procedure—Proceed as directed for *Method II*, but place in the flask only 25 mL of the liquid to be tested.

Method II

Apparatus—Use an apparatus consisting of the following parts:

Distilling Flask—A round-bottom distilling flask, of heat-resistant glass, of 200-mL capacity, and having a total length of 17 to 19 cm, and an inside neck diameter of 20 to 22 mm. Attached about midway on the neck, approximately 12 cm from the bottom of the flask, is a side-arm 10 to 12 cm long and 5 mm in internal diameter, which forms an angle of 70° to 75° with the lower portion of the neck.

Condenser—A straight glass condenser 55 to 60 cm in length with a water jacket about 40 cm in length, or a condenser of other design having equivalent condensing capacity. The lower end of the condenser may be bent to provide a delivery tube, or it may be connected to a bent adapter that serves as a delivery tube.

Insulating Boards—Two pieces of insulating board, 5 to 7 mm thick and 14 to 16 cm square, suitable for confining the heat to the lower part of the flask. Each board has a hole in its center, and the two boards differ only with respect to the diameter of the hole, i.e., the diameters are 4 cm and 10 cm, respectively. In use, the boards are placed one upon the other, and rest on a tripod or other suitable support, with the board having the larger hole on top.

Receiver—A 100-mL cylinder graduated in 1-mL subdivisions.

Thermometer—In order to avoid the necessity for an emergent stem correction, an accurately standardized, partial-immersion thermometer having the smallest practical subdivisions (not greater than 0.2°) is recommended. Suitable thermometers are available as the ASTM E-1 series 37C through 41C, and 102C through 107C (see *Thermometers* ⟨21⟩). When placed in position, the stem is located in the center of the neck, and the top of the contraction chamber (or bulb, if 37C or 38C is used) is level with the bottom of the outlet to the side-arm.

Heat Source—A small Bunsen burner or an electric heater or mantle capable of adjustment comparable to that possible with a Bunsen burner.

Procedure—Assemble the apparatus, and place in the flask 100 mL of the liquid to be tested, taking care not to allow any of the liquid to enter the side-arm. Insert the thermometer, shield the entire burner and flask assembly from external air currents, and apply heat, regulating it so that between 5 and 10 minutes elapse before the first drop of distillate falls from the condenser. Continue the distillation at a rate of 4 to 5 mL of distillate per minute, collecting the distillate in the receiver. Note the temperature when the first drop of distillate falls from the condenser, and again when the last drop of liquid evaporates from the bottom of the flask or when the specified percentage has distilled over. Unless otherwise specified in the individual monograph, apply when necessary the emergent stem correction and report the temperatures adjusting the barometric pressure by the following formula:

$$t = t_0 + [(t_0 10^{-4} + 0.033)(760 - p)]$$

in which t is the corrected boiling temperature, in Celsius scale; t_0 is the measured boiling temperature, in Celsius scale; and p is the barometric pressure at the time of measurement, in mm Hg.

⟨724⟩ DRUG RELEASE

This test is provided to determine compliance with drug-release requirements where specified in individual monographs. Use the apparatus specified in the individual monograph. Replace the aliquots withdrawn for analysis with equal volumes of fresh *Dissolution Medium* at the temperature specified in the monograph or, where it can be shown that replacement of the medium is not necessary, correct for the volume change in the calculation. Keep the vessel covered for the duration of the test, and verify the temperature of the mixture under test at suitable times.

TRANSDERMAL DELIVERY SYSTEMS—GENERAL DRUG RELEASE STANDARDS

Apparatus 5 (Paddle over Disk)

Apparatus—Use the paddle and vessel assembly from *Apparatus 2* as described under *Dissolution* ⟨711⟩, with the addition of a stainless steel disk assembly[1] designed for holding the transdermal system at the bottom of the vessel. Other appropriate devices may be used, provided they do not sorb, react with, or interfere with the specimen being tested[2]. The temperature is maintained at 32 ± 0.5°. A distance of 25 ± 2 mm between the paddle blade and the surface of the disk assembly is maintained during the test. The vessel may be covered during the test to minimize evaporation. The disk assembly for holding the transdermal system is designed to minimize any "dead" volume between the disk assembly and the bottom of the vessel. The disk assembly holds the system flat and is positioned such that the release surface is parallel with the bottom of the paddle blade (see *Figure 1*).

[1] Disk assembly (stainless support disk) may be obtained from Millipore Corp., Ashley Rd., Bedford, MA 01730.

[2] A suitable device is the watchglass-patch-polytef mesh sandwich assembly available as the Transdermal Sandwich™ from Hanson Research Corp., 9810 Variel Ave., Chatsworth, CA 91311.

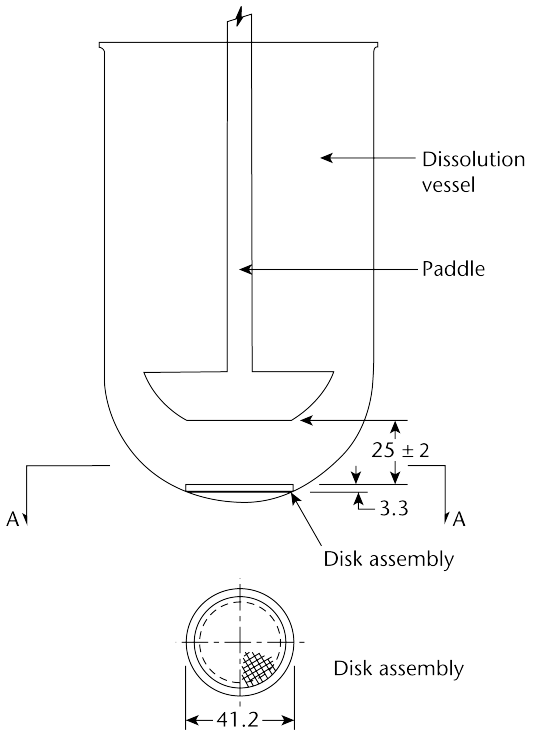


Figure 1. Paddle over disk.
(All measurements are expressed in mm unless noted otherwise.)

Apparatus Suitability Test and **Dissolution Medium**—Proceed as directed for *Apparatus 2* under *Dissolution* ⟨711⟩.

Procedure—Place the stated volume of the *Dissolution Medium* in the vessel, assemble the apparatus without the disk assembly, and equilibrate the medium to 32 ± 0.5°. Apply the transdermal system to the disk assembly, assuring that the release surface of the system is as flat as possible. The system may be attached to the disk by applying a suitable adhesive[3] to the disk assembly. Dry for 1 minute. Press the system, release surface side up, onto the adhesive-coated side of the disk assembly. If a membrane[4] is used to support the system, it is applied so that no air bubbles occur between the membrane and the release surface. Place the disk assembly flat at the bottom of the vessel with the release surface facing up and parallel to the edge of the paddle blade and surface of the *Dissolution Medium.* The bottom edge of the paddle is 25 ± 2 mm from the surface of the disk assembly. Immediately operate the apparatus at the rate specified in the monograph. At each sampling time interval, withdraw a specimen from a zone midway between the surface of the *Dissolution Medium* and the top of the blade, not less than 1 cm from the vessel wall. Perform the analysis on each sampled aliquot as directed in the individual monograph, correcting for any volume losses, as necessary. Repeat the test with additional transdermal systems.

Time—The test time points, generally three, are expressed in hours. Specimens are to be withdrawn within a tolerance of ±15 minutes or ±2% of the stated time, the tolerance that results in the narrowest time interval being selected.

Interpretation—Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient released from the system conform to *Acceptance Table 1* for transdermal drug delivery systems. Continue testing through the three levels unless the results conform at either L_1 or L_2.

Acceptance Table 1

Level	Number Tested	Criteria
L_1	6	No individual value lies outside the stated range.
L_2	6	The average value of the 12 units (L_1 + L_2) lies within the stated range. No individual value is outside the stated range by more than 10% of the average of the stated range.
L_3	12	The average value of the 24 units (L_1 + L_2 + L_3) lies within the stated range. Not more than 2 of the 24 units are outside the stated range by more than 10% of the average of the stated range; and none of the units is outside the stated range by more than 20% of the average of the stated range.

Apparatus 6 (Cylinder)

Apparatus—Use the vessel assembly from *Apparatus 1* as described under *Dissolution* ⟨711⟩, except to replace the basket and shaft with a stainless steel cylinder stirring element and to maintain the temperature at 32 ± 0.5° during the test. The shaft and cylinder components of the stirring element are fabricated of stainless steel to the specifications shown in *Figure 2*. The dosage unit is placed on the cylinder at the beginning of each test. The distance between the inside bottom of the vessel and the cylinder is maintained at 25 ± 2 mm during the test.

Dissolution Medium—Use the medium specified in the individual monograph (see *Dissolution* ⟨711⟩).

Procedure—Place the stated volume of the *Dissolution Medium* in the vessel of the apparatus specified in the individual monograph, assemble the apparatus, and equilibrate the *Dissolution Medium* to 32 ± 0.5°. Unless otherwise directed in the individual monograph, prepare the test system prior to test as follows. Remove the protective liner from the system, and place the adhesive side on a piece of Cuprophan[4] that is not less than 1 cm larger on all sides than the system. Place the system, Cuprophan covered side down, on a clean surface, and apply a suitable adhesive[3] to the exposed Cuprophan borders. If necessary, apply additional adhesive to the back of the system. Dry for 1 minute. Carefully apply the adhesive-coated side of the system to the exterior of the cylinder such that the long axis of the system fits around the circumference of the cylinder. Press the Cuprophan covering to remove trapped air bubbles. Place the cylinder in the apparatus, and immediately rotate at the rate specified in the individual monograph. Within the time interval specified, or at each of the times stated, withdraw a quantity of *Dissolution Medium* for analysis from a zone midway between the surface of the *Dissolution Medium* and the top of the rotating cylinder, not less than 1 cm from the vessel wall. Perform the analysis as directed in the individual monograph, correcting for any volume losses as necessary. Repeat the test with additional transdermal drug delivery systems.

Time—Proceed as directed under *Apparatus 5.*

Interpretation—Unless otherwise specified in the individual monograph, the requirements are met if the quantities of active ingredient released from the system conform to *Acceptance Table 1* for transdermal drug delivery systems. Continue testing through the three levels unless the results conform at either L_1 or L_2.

[3] Use Dow Corning, MD7-4502 Silicone Adhesive 65% in ethyl acetate, or the equivalent.
[4] Use Cuprophan, Type 150 pm, 11 ± 0.5-µm thick, an inert, porous cellulosic material, which is available from Medicell International Ltd., 239 Liverpool Road, London NI ILX, England.

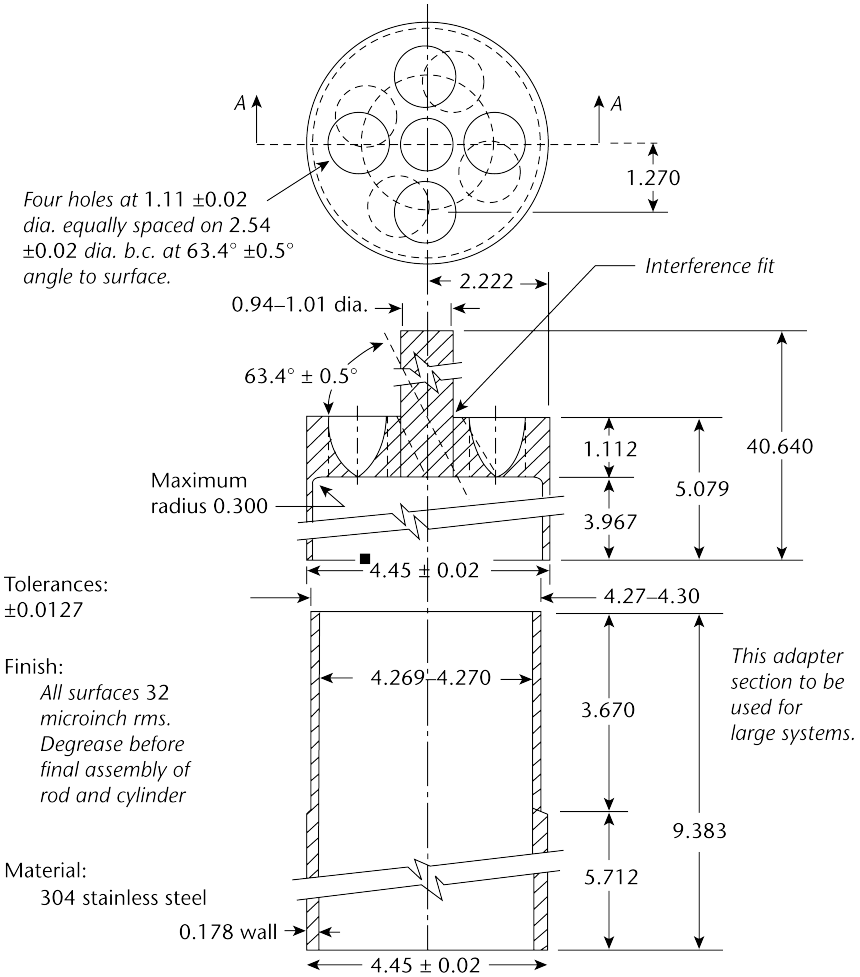
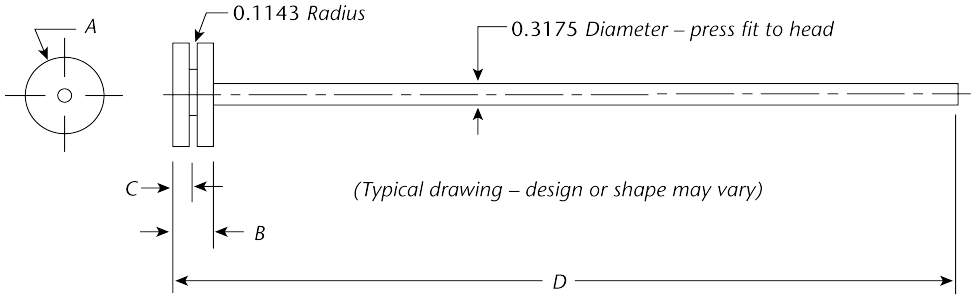


Figure 2. Cylinder stirring element.[5]
(All measurements are expressed in cm unless noted otherwise.)

Apparatus 7 (Reciprocating Holder)

NOTE—This apparatus may also be specified for use with a variety of dosage forms.

[5]The cylinder stirring element is available from Accurate Tool, Inc., 25 Diaz St., Stamford, CT 06907, or from VanKel Technology Group, 13000 Weston Parkway, Cary, NC 27513.



Dimensions are in centimeters

	HEAD				ROD		O-RING
System[a]	*A (Diameter)*	*B*	*C*	Material[b]	*D*	Material[c]	*(not shown)*
1.6 cm^2	1.428	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2–113–V884–75
2.5 cm^2	1.778	0.9525	0.4750	SS/VT	30.48	SS/P	Parker 2–016–V884–75
5 cm^2	2.6924	0.7620	0.3810	SS/VT	8.890	SS/P	Parker 2–022–V884–75
7 cm^2	3.1750	0.7620	0.3810	SS/VT	30.48	SS/P	Parker 2–124–V884–75
10 cm^2	5.0292	0.6350	0.3505	SS/VT	31.01	SS/P	Parker 2–225–V884–75

[a] Typical system sizes.

[b] SS/VT=Either stainless steel or virgin Teflon.

[c] SS/P=Either stainless steel or Plexiglas.

Figure 3. Reciprocating disk sample holder.[7]

Apparatus—The assembly consists of a set of volumetrically calibrated or tared solution containers made of glass or other suitable inert material[6], a motor and drive assembly to reciprocate the system vertically and to index the system horizontally to a different row of vessels automatically if desired, and a set of suitable sample holders (see *Figure 3*[7] and *Figures 4a–4d*). The solution containers are partially immersed in a suitable water bath of any convenient size that permits maintaining the temperature, T, inside the containers at 32 ± 0.5° or within the allowable range, as specified in the individual monograph, during the test. No part of the assembly, including the environment in which the assembly is placed, contributes significant motion, agitation, or vibration beyond that due to the smooth, vertically reciprocating sample holder. Apparatus that permits observation of the system and holder during the test is preferable. Use the size

[6] The materials should not sorb, react with, or interfere with the specimen being tested.

[7] The reciprocating disk sample holder may be purchased from ALZA Corp., 1900 Charleston Road, P.O. Box 7210, Mt. View, CA 94039–7210 or VanKel Technology Group.

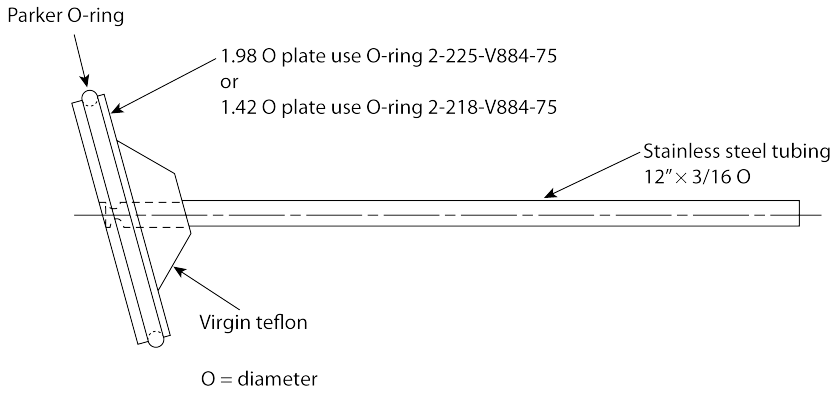


Figure 4a. Transdermal system holder—angled disk.

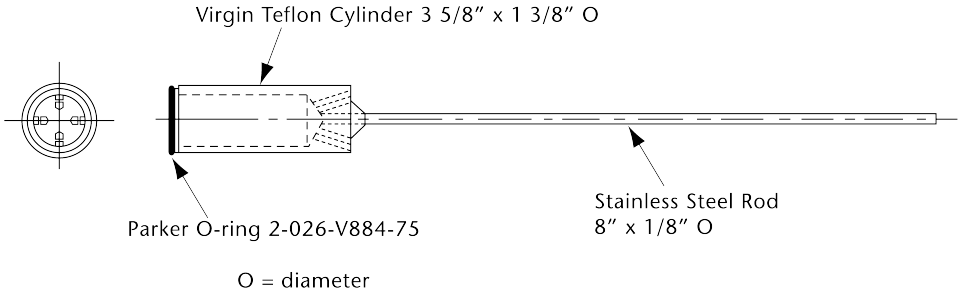


Figure 4b. Transdermal system holder—cylinder.

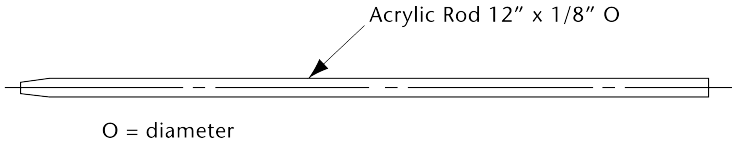
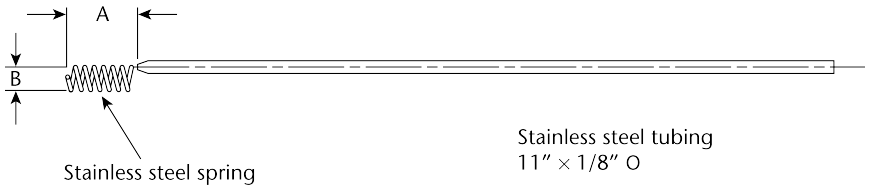


Figure 4c. Oral extended-release tablet holder—rod, pointed for gluing.



Stainless steel spring Dimensions

A	B
1.45	.58 O
1.40	.31 O
.96	.33 O
.60	.25 O

O = diameter

Figure 4d. Oral extended-release tablet holder—spring holder.

container and sample holder as specified in the individual monograph.

Dissolution Medium—Use the *Dissolution Medium* specified in the individual monograph (see *Dissolution* ⟨711⟩).

Sample Preparation A (Coated tablet drug delivery system)—Attach each system to be tested to a suitable sample holder (e.g., by gluing system edge with 2-cyano acrylate glue onto the end of a plastic rod or by placing the system into a small nylon net bag at the end of a plastic rod or within a metal coil attached to a metal rod).

Sample Preparation B (Transdermal drug delivery system)—Press the system onto a dry, unused piece of Cuprophan[4], nylon netting, or equivalent with the adhesive side against the selected substrate, taking care to eliminate air bubbles between the substrate and the release surface. Attach the system to a suitable sized sample holder with a

suitable O-ring such that the back of the system is adjacent to and centered on the bottom of the disk-shaped sample holder or centered around the circumference of the cylindrical-shaped sample holder. Trim the excess substrate with a sharp blade.

Sample Preparation C (Other drug delivery systems)—Attach each system to be tested to a suitable holder as described in the individual monograph.

Procedure—Suspend each sample holder from a vertically reciprocating shaker such that each system is continuously immersed in an accurately measured volume of *Dissolution Medium* within a calibrated container pre-equilibrated to temperature, T. Reciprocate at a frequency of about 30 cycles per minute with an amplitude of about 2 cm, or as specified in the individual monograph, for the specified time in the medium specified for each time point. Remove the solution containers from the bath, cool to room temperature, and add sufficient solution (i.e., water in most cases) to correct for evaporative losses. Perform the analysis as directed in the individual monograph. Repeat the test with additional drug delivery systems as required in the individual monograph.

Interpretation—Unless otherwise specified in the individual monograph, the requirements are met if the quantities of the active ingredients released from the system conform to *Acceptance Table 2* under *Dissolution* ⟨711⟩ for coated tablet drug delivery systems, to *Acceptance Table 1* for transdermal drug delivery systems, or as specified in the individual monograph. Continue testing through the three levels unless the results conform at either L_1 or L_2.

⟨726⟩ ELECTROPHORESIS

Electrophoresis refers to the migration of electrically charged proteins, colloids, molecules, or other particles when dissolved or suspended in an electrolyte through which an electric current is passed.

Based upon the type of apparatus used, electrophoretic methods may be divided into two categories, one called *free solution* or moving boundary electrophoresis and the other called *zone electrophoresis*.

In the *free solution* method, a buffered solution of proteins in a U-shaped cell is subjected to an electric current which causes the proteins to form a series of layers in order of decreasing mobility, which are separated by boundaries. Only a part of the fastest moving protein is physically separated from the other proteins, but examination of the moving boundaries using a schlieren optical system provides data for calculation of mobilities and information on the qualitative and quantitative composition of the protein mixture.

In *zone electrophoresis*, the sample is introduced as a narrow zone or spot in a column, slab, or film of buffer. Migration of the components as narrow zones permits their complete separation. Remixing of the separated zones by thermal convection is prevented by stabilizing the electrolyte in a porous matrix such as a powdered solid, or a fibrous material such as paper, or a gel such as starch, agar, or polyacrylamide.

Various methods of zone electrophoresis are widely employed. *Gel electrophoresis*, particularly the variant called *disk electrophoresis*, is especially useful for protein separation because of its high resolving power.

Gel electrophoresis, which is employed by the compendium, is discussed in more detail following the presentation of some theoretical principles and methodological practices, which are shared in varying degrees by all electrophoretic methods.

The electrophoretic migration observed for particles of a particular substance depends on characteristics of the particle, primarily its electrical charge, its size or molecular weight, and its shape, as well as characteristics and operating parameters of the system. These latter include the pH, ionic strength, viscosity and temperature of the electrolyte, density or cross-linking of any stabilizing matrix such as gel, and the voltage gradient employed.

Effect of Charge, Particle Size, Electrolyte Viscosity, and Voltage Gradient—Electrically charged particles migrate toward the electrode of opposite charge, and molecules with both positive and negative charges move in a direction dependent on the net charge. The rate of migration is directly related to the magnitude of the net charge on the particle and is inversely related to the size of the particle, which in turn is directly related to its molecular weight.

Very large spherical particles, for which Stokes' law is valid, exhibit an electrophoretic mobility, u_0, which is inversely related to the first power of the radius as depicted in the equation:

$$u_0 = v/E = Q/6\pi r\eta$$

where v is the velocity of the particle, E is the voltage gradient imposed on the electrolyte, Q is the charge on the particle, r is the particle radius, and η is the viscosity of the electrolyte. This idealized expression is strictly valid only at infinite dilution and in the absence of a stabilizing matrix such as paper or a gel.

Ions, and peptides up to molecular weights of at least 5000, particularly in the presence of stabilizing media, do not obey Stokes' law, and their electrophoretic behavior is best described by an equation of the type:

$$u_0 = Q/A\pi r^2\eta$$

where A is a shape factor generally in the range of 4 to 6, which shows an inverse dependence of the mobility on the square of the radius. In terms of molecular weight, this implies an inverse dependence of mobility on the $^2/_3$ power of the molecular weight.

Effect of pH—The direction and rate of migration of molecules containing a variety of ionizable functional groups, such as amino acids and proteins, depends upon the pH of the electrolyte. For instance, the mobility of a simple amino acid such as glycine varies with pH approximately as shown in *Figure 1.*

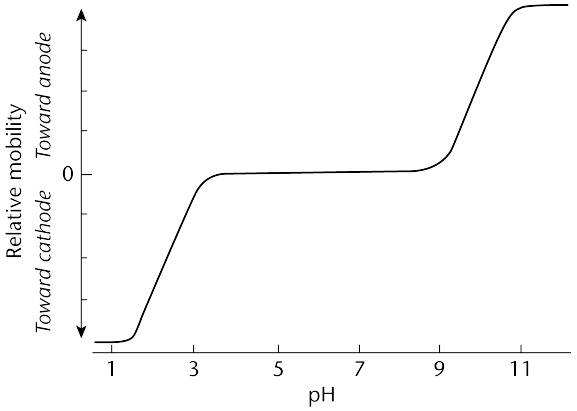


Figure 1

The pK_a values of 2.2 and 9.9 coincide with the inflection points of the sigmoid portions of the plot. Since the respective functional groups are 50% ionized at the pH values where $pH = pK_a$, the electrophoretic mobilities at these points are half of the value observed for the fully ionized