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PHYSICAL CHEMISTRY

FOR

STUDENTS OF BIOLOGY AND MEDICINE

BY

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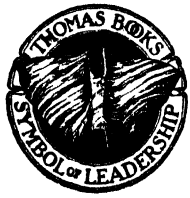
SECOND EDITION

(WITH LABORATORY DIRECTIONS)



ıNGFIELD, ILLINOIS BALTIMORE, MARYLAND

CHARLES C THOMAS

1934

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PREFACE

The importance of physical chemistry as a basis for the explanation of fundamental physiological phenomena has long been recognized by research workers in the biological sciences. An appreciation of modern biological research often involves an understanding of the language of physical chemistry. It is hoped that the present work may help students to gain such a reading knowledge of the science. The book deals with only a small part of the field of physical chemistry; the selection of topics has been guided by their past application in biological work. For a thorough mastery of the field, which is essential for any one who expects to use physical chemistry as a research tool, students are urged to take the excellent courses offered in university departments of chemistry, which use much more comprehensive textbooks. References to a few such texts have been put at the ends of the chapters, in the hope that the ambitious student will by no means confine his reading to this book. In those chapters where recent developments are discussed, reference is made to more specialized monographs and to journal articles, not with the idea that any one student will look up all of the references, but simply as a convenience for those who may wish to read further on some one topic.

The material presented in this book has been offered during the past five years to medical and graduate students in Yale University as a part of the course in physiology. The study of theoretical principles has been accompanied by simple laboratory experiments of a semi-quantitative nature. In order that the laboratory course may be kept elastic and constantly improved, it has seemed advisable not to include laboratory directions in this book.

The writer is indebted to Dr. John F. Fulton for encouraging him to write the book, to Drs. R. S. Anderson, N. F. Burk, H. E. Himwich, L. F. Nims, and J. P. Peters for reading various parts of the manuscript, to Dr. Nims for making drawings, and to Mrs. J. P. Peters for arranging the bibliography. He is fur-

ther indebted to his former teacher, Dr. John M. Nelson, of the Department of Chemistry of Columbia University, and to the late Dr. Jacques Loeb, of the Rockefeller Institute for Medical Research, for an introduction into the ways in which biological science can be advanced by the use of the quantitative methods of physical chemistry.

D.I.H.

New Haven
July, 1932

PREFACE TO SECOND EDITION

The principal change which has been made in this book in the inclusion of laboratory directions. The experiments have been adapted from various sources which are acknowledged in the references. The attempt has been made to keep the apparatus as simple as possible. Additional experiments, which have been given to a few advanced students, include the electrometric measurement of pH and of oxidation-reduction potentials, and some of Loeb's experiments on membrane equilibria.

Other changes include the addition of sections on Brønsted's conception of acids and bases, and on surface films. The bibliography has been revised and somewhat expanded, as a result of the firm conviction that students of science ought to be encouraged to read the original literature.

The subject matter of this book is now taught at Yale in a separate elective course, instead of as a part of the course in physiology. This change was suggested by Dr. Fulton, and the writer feels that it has worked well. Although the number of students has been considerably smaller, the quality of their work has improved greatly.

The writer wishes to acknowledge his indebtedness to Dr. Leslie F. Nims for his help in selecting and testing experiments, and to Mr. Charles C Thomas for his friendly coöperation in bringing out the book.

D.I.H.

New Haven
May, 1934

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PHYSICAL CHEMISTRY

FOR

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CHAPTER I

GASES

Boyle's Law. The variation of the volume of a fixed amount of gas with the pressure exerted on it was studied by Robert Boyle[1] about 1661. One form of the apparatus used by him consisted of a long bent tube, sealed at one end. The bend of the tube was filled with mercury, enclosing air in the sealed end. The volume of this air was measured first when the mercury levels were the same, as at a and b, Fig. 1. Under these conditions the enclosed gas was under the pressure of the atmosphere. Additional mercury was poured into the open end of the tube, causing both levels to rise, but not changing the amount or mass of the enclosed gas. The difference between the mercury levels at any time measured the difference in pressure between the enclosed gas and the atmosphere, and the volume of the gas could be obtained from the length of the tube occupied by it. When the levels were such that the gas occupied half its original volume (a' and b', Fig. 1), Boyle found that the difference in mercury levels was equal to the height of the mercury in a barometer, indicating that the gas was now under a pressure of two atmospheres. From such measurements with air and other gases, it has been found that *when a fixed mass of gas is kept at constant temperature, its volume varies inversely as the pressure exerted on or by it.* This is Boyle's (or Mariotte's) law. It may be expressed by the equation

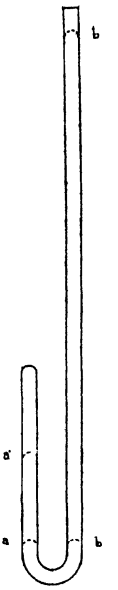

FIG. 1.
Boyle's Tube

$$v = \frac{k}{p} \quad (m = \text{constant}, t = \text{constant}) \qquad (1)$$

[1] The name of Robert Boyle (1627–1691) is noteworthy not only because of his discovery of this law, but because of his influence on the progress of science. He was one of the founders of the organization which later became the Royal Society of London, and was a powerful advocate of the experimental method in science at a time when scholars did more philosophizing than experimenting.

where p refers to pressure, v to volume, m to mass, t to temperature, and k is a constant of proportionality.

Charles's Law. In 1802 the French chemist Gay-Lussac[2] published the results of experiments on the expansion of air and other gases with rise in temperature. He found that every gas examined expanded by the same fraction of its volume at 0° when the temperature was raised to 100° and the pressure was kept at 1 atmosphere. Gay-Lussac stated in this paper that Charles,[3] a French physicist, had worked about fifteen years earlier on the effect of temperature on the pressure of gases confined at approximately constant volume. Charles had not published his results. Since by Boyle's law the product of pressure and volume is constant, a change in temperature must have the same effect on either pressure or volume if the other factor is held constant. Thus Charles and Gay-Lussac were really studying the same thing, although their methods differed. The generalization of their results is usually known as Charles's Law.

Gay-Lussac concluded that since each gas expanded by 37.5 per cent when heated from 0° to 100°, the expansion per degree C. was 0.375/100 or 1/266.7. More precise studies by subsequent workers have indicated that this figure approaches 1/273.1 for gases at very low pressures and high temperatures. Hence the law of Charles or Gay-Lussac may be stated as follows: *If the same mass of gas is kept at constant pressure, its volume increases by* 1/273.1 *of that at* 0°C. *for a rise in temperature of* 1°C. This law may also be stated by the equation

$$v = v_0\left(1 + \frac{t}{273.1}\right) \quad (m = \text{constant}, p = \text{constant}) \qquad (2)$$

where v_0 is the volume at 0°C. and v is the volume at any temperature t°C.

[2] Joseph Louis Gay-Lussac (1778–1850), of Paris, was only twenty-four when this paper was published. He later discovered the law of the combination of gases by volume, which is more commonly associated with his name. Both he and Charles were among the first to make balloon ascensions to study the chemistry and physics of the atmosphere.

[3] Jacques Alexandre César Charles (1746-1823) was Professor of Physics at the Conservatoire des Arts et Métiers in Paris. He was the first to suggest the use of hydrogen for filling balloons.

force per unit area, the quantity pv has the dimensions of force $\times$ volume $\div$ area, or force $\times$ length, which is work. Hence R must represent work or energy $\div$ temperature. This means that its dimensions, in the fundamental physical quantities of mass, length, and time, are $ML^2T^{-2} \div$ temperature. The value of R derived in equation (7) is 0.08206 liter-atmospheres per degree. In other common units R has the values 1.9869 calories per degree, or 8.313 joules per degree.

Diffusion of Gases. If a small glass bulb containing bromine is broken in the bottom of a large bottle of air, the brown vapor of the bromine can be seen to spread rapidly throughout the whole available space. In this case the motion of the bromine vapor takes place in spite of the action of gravity, for bromine is heavier than air. All gases are freely miscible in all proportions, and they mix spontaneously and rapidly, so that samples of a gas mixture taken from different parts of a vessel have exactly the same composition. Diffusion is the process whereby the spontaneous mixing of gases takes place, so that each gas is distributed equally throughout all the available space.

Diffusion always takes place from a region of higher concentration to one of lower, so that the result is an equalization of concentrations. The process is accelerated by rise in temperature. The rates of diffusion of different gases may be compared by slowing down the process by means of a porous septum, or by comparing the times of effusion or outflow of equal volumes through a narrow capillary tube under the same difference in pressure. Such experiments led Graham,[6] about 1831, to the generalization that *the rates of diffusion of different gases are inversely proportional to the square roots of their densities.* Avogadro's law implies that the densities of gases are proportional to their molecular weights. Hence Graham's law of diffusion is contained in the statement that the *rates of diffusion of gases are inversely proportional to the square roots of their molecular weights*

[6] Thomas Graham (1805–1869), who is often called the father of colloid chemistry, taught in Glasgow and London, and later held (as had Isaac Newton) the office of Master of the Mint. He studied diffusion in liquids as well as in gases, and discovered the process of dialysis.

or

$$\frac{D_1}{D_2} = \sqrt{\frac{M_2}{M_1}} \tag{9}$$

where D refers to rate of diffusion and M to molecular weight.

Dalton's Law of Partial Pressures. The pressure of a mixture of gases may be considered to be the sum of several smaller pressures, each of which is ascribed to a single kind of gas in the mixture. Dalton[7] pointed out, about 1800, that the observed value of the total pressure is correctly given by adding up a series of partial pressures, each calculated for a single gas by means of the perfect gas law as if the single gas occupied all of the available volume and the other gases were not there. This relation applies, of course, only to mixtures of gases which do not react chemically with one another, and it holds exactly in this form only within the same limits as the perfect gas law for a single gas. Dalton's law, then, states that *the pressure of a mixture of gases for which the perfect gas law holds is equal to the sum of the partial pressures calculated by assuming that each gas has the same pressure as if it alone were present in the total volume of the mixture.*

This law may be expressed by the equation

$$p = \frac{N_A RT}{v} + \frac{N_B RT}{v} + \frac{N_C RT}{v} + \cdots \tag{10}$$

where p is the total pressure and v the total volume of the mixture, and N_A, N_B, etc., are the numbers of moles of the gases A, B, etc., present. Each term in equation (10) represents the partial pressure of a single gas, calculated according to Dalton; that is,

$$p_A = \frac{N_A RT}{v}, \qquad p_B = \frac{N_B RT}{v}, \qquad \text{etc.,} \tag{11}$$

where v is again the total volume of the mixture.

[7] The name of John Dalton (1766–1844) is familiar in connection with the atomic theory. He was a Quaker schoolmaster of Manchester, England, who continued to tutor small boys even after he had acquired a European reputation as a scientific thinker of the first rank.

Since the total number, N, of moles of gas in the mixture is equal to the sum of the numbers of moles of the individual gases, or

$$N = N_A + N_B + N_C + \cdots, \tag{12}$$

equation (10) may be rewritten

$$p = \frac{NRT}{v}, \tag{13}$$

which is simply a statement of the condition that the perfect gas law applies to the mixture as a whole. By combining equations (11) and (13) we get

$$p_A = \frac{N_A}{N} p, \qquad p_B = \frac{N_B}{N} p, \qquad \text{etc.,} \tag{14}$$

which is an alternative definition of partial pressure: *the partial pressure of a gas in a mixture is equal to the product of its mole fraction and the total pressure.*

It should be possible to test Dalton's law if a membrane could be obtained permeable to one gas in a mixture but not to others. This has been done in the case of gas mixtures containing hydrogen, for metallic palladium at high temperatures is permeable to hydrogen but not to other gases such as nitrogen. If a palladium vessel containing nitrogen at a sufficiently high temperature is surrounded by an atmosphere of hydrogen held in an outer jacket with rigid impervious walls, it is found that the final pressure inside the palladium vessel is equal to the sum of the original pressure of the nitrogen and the final pressure of the outside atmosphere of hydrogen. This means that at equilibrium the partial pressure of hydrogen inside the vessel is equal to the pressure of the hydrogen outside, and that the partial pressures of the hydrogen and nitrogen in the resulting mixture inside the vessel are additive, as Dalton's law requires.

The Kinetic Theory of Gases. The various laws of the behavior of gases, which have been stated in the preceding paragraphs as the result of experimental findings, can all be deduced from the kinetic theory of gases. While these deductions are too advanced to be considered here, a brief statement of the ele-

ments of this important theory may be helpful. According to the kinetic theory a gas consists of perfectly elastic molecules which are continually in rapid and chaotic or unordered motion. This motion is an expression of the heat energy of the gas, and its velocity increases with rise in temperature. The molecules move in straight lines, but the direction of motion is changed very frequently by collisions with other molecules or with the walls of the containing vessel. This molecular motion accounts at once, without further assumptions, for the facts of diffusion. The pressure exerted by a gas results from the continual bombardment of the walls of the containing vessel by the rapidly moving molecules. Hence the partial pressure of a gas in a mixture should be proportional to the mole fraction of that gas, as it is, and the total pressure must obviously be the sum of all the partial pressures.

In order to deduce the perfect gas law, two additional assumptions are necessary. These are that the volume occupied by the molecules themselves is negligible with respect to the total volume occupied by the gas, and that the molecules exert no attractive forces on one another. The deviations from the law of perfect gases shown by real gases are explained by the fact that these two assumptions are not strictly true. Equations such as that of van der Waals, which describe more exactly the behavior of real gases, contain additional terms involving the volume of the gas molecules and the attractive forces between them.

Physiological Applications of the Gas Laws. Probably the most common use of the gas laws in practical physiological work is in correcting the volume of a gas, as measured in the laboratory, to standard conditions. If the gas has been in contact with water it will be saturated with water vapor. According to Dalton's law the partial pressure of the gas will be the total pressure, minus the partial pressure of the water vapor. (The latter figure, for the temperature of the experiments, can be obtained from tables.) The observed volume under this partial pressure is then corrected to standard conditions by Boyle's and Charles's laws.

Studies of respiration involve a knowledge of the laws of partial pressure and of diffusion, as well as an understanding of the factors governing the solubility of gases in liquids. The latter will be discussed in another chapter.

PROBLEMS

1. Devise a simple method for testing Charles's law in the laboratory.

2. Invent a set of data which agree with the laws of Boyle and Charles, and plot them to show graphically the relation expressed in each of these laws.

3. Calculate the volume occupied by one mole of a perfect gas at 20°C. and at pressures varying from 0.1 to 1.0 atmosphere in increments of 0.1 atmosphere. Plot the results. Will the curve ever cross the line of zero pressure or of zero volume? What is the mathematical name of such a curve?

4. What sort of a curve would be obtained by plotting the values of pv in Problem 3 against pressure? Against volume? Plot the values of pv for 1 mole of a perfect gas against temperature at 50° intervals from $-250°$ to $+100°$C. Read from this curve the value of pv at the absolute zero of temperature. What is the slope of this curve?

5. Show the relation between the perfect gas law and the equation

$$\frac{pv}{N} = 22.4 + 0.082t.$$

6. A sample of expired air was passed through suitable aqueous solutions to absorb CO_2 and O_2. Its volume, as measured over water, was found to be 23.4 cc. at 18°C. The barometer reading was 753 mm. Hg. Calculate the volume of this gas under standard conditions. How many moles did it contain? What was its weight?

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127. WASHBURN, Chapters I–II.

* In this and all subsequent chapters the numbers refer to the complete bibliography at the end of the book, where full titles may be found.

CHAPTER II

Liquids and Gases

General Properties of Liquids. Both gases and liquids are classified as fluids, which means that they flow readily, having no definite shape. Whereas a gas expands freely, so that it occupies the whole of any available space, a liquid has a definite volume at any given temperature. While the volume of a liquid does depend on the pressure and temperature, alterations in these conditions have much less effect than in the case of gases. A liquid is but little compressed by tremendous pressure; and while liquids do, in general, expand with rise in temperature, the resulting volume change is of a much lower order of magnitude than in the case of gaseous expansion. The uniformity found in the behavior of gases is lacking in the case of liquids; each liquid has its own individual coefficients of thermal expansion and of compressibility. The most common liquid, water, actually decreases in volume as the temperature is raised from 0° to 4°C., expanding with rise in temperature only above 4°C.

Matter in the liquid state is much more concentrated than the same matter in the gaseous state under comparable conditions. Thus 1 mole (18 grams) of liquid water at 1 atmosphere and 100°C. occupies only about 18.8 cc., while the same quantity of gaseous water or steam under the same conditions occupies more than 30 liters.

On the basis of the kinetic theory these properties are explained by assuming a pure liquid to consist of moving molecules like gas molecules, but much closer together, and hence more influenced by their mutual attractive or cohesive forces. Accordingly their average velocity is less, as is their mean free path, or the average distance which a molecule can move before colliding with another.

Vaporization of Liquids. If a pure liquid such as benzene is poured into an open dish and left in the air at a temperature well below its boiling point, the liquid eventually disappears

as a result of evaporation. The presence of gaseous benzene in the surrounding air is readily detected by its odor. If the temperature of the liquid is taken at intervals during this process of spontaneous evaporation, the temperature is found to decrease. These facts are explained, on the basis of the kinetic theory, by assuming that certain molecules in the liquid have velocities sufficiently high so that they are projected up through the surface and out into the gas phase above the liquid. As soon as they cross the boundary, the liquid molecules become gas molecules. There is, in general, no intrinsic difference between molecules of the same substance in the two states except that of average kinetic energy or velocity. Since the temperature of the liquid is a measure of the average kinetic energy of the molecules remaining in it, the temperature must become lower as the faster molecules, which have greater kinetic energy, leave the liquid. Similar considerations apply to any liquid. In the extreme case of mercury, evaporation into the air may be rather difficult to detect in ordinary periods of time, but theoretically its behavior is similar to that of benzene except for a marked difference in the rate of evaporation under ordinary laboratory conditions.

Vapor Pressure. If a little benzene is poured into the bottom of a large empty bottle open to the air, and the bottle is then stoppered, evaporation proceeds only to a limited extent. The air in the bottle becomes saturated with benzene vapor, but since the vessel is closed this gaseous benzene is unable to diffuse away and evaporation appears to stop. This apparently stationary state is explained as a case of dynamic equilibrium. Molecules of benzene are leaving the liquid all the time, but in any given time an equal number of gaseous benzene molecules is passing the interface in the opposite direction and condensing or becoming molecules of liquid. Since the heat changes are equal and opposite, the temperature of the benzene, after equilibrium has been reached, shows no further spontaneous change.

In such a system consisting of a liquid and its vapor in equilibrium, the vapor exerts pressure, just as any other gas does.

At any given temperature the partial pressure of this vapor is a fixed and definite quantity for each substance, provided always that there is an excess of the liquid present to keep the available space saturated with the vapor. The pressure of the vapor in equilibrium with any liquid at constant temperature is constant, depending only on the nature of the substance and the temperature, but independent of the actual amounts of the liquid and vapor present, the presence or absence of other gases, and the total pressure. The relation of vapor pressure to total gas pressure is included in Dalton's law of partial pressures.

The existence of a definite vapor pressure of water, for example, may be demonstrated as follows. A long tube, sealed at one end, is filled with mercury and inverted without the introduction of any air so that its open end is beneath the surface of mercury in a dish open to the air. If the tube is more than 76 cm. in length, the mercury falls away from the sealed end until the difference in mercury levels corresponds to the pressure of the atmosphere. This is the well-known experiment of Torricelli[1] (1643). The space in the end of the tube above the mercury is a Torricellian vacuum; if the experiment is properly done, it contains no gas except an infinitesimal amount of mercury vapor. If a little water is released in the mercury below the open end of the tube, it rises to the top of the mercury column, which at once falls through a distance corresponding to the vapor pressure of water at the temperature of the experiment.

Exact measurements of the vapor pressures of water and other liquids have been carried out at various temperatures by more refined methods, and the results may be found in tables of physical constants. In the case of water, the vapor pressure is often called aqueous tension. Values of this quantity are used in correcting measured volumes of moist gases to standard conditions.

[1] Evangelista Torricelli (1608–1647), who succeeded Galileo as Professor of Mathematics in Florence, is said to have devised this experiment, which was actually carried out by a young pupil named Viviani. This work led the French scientist Pascal to have the experiment repeated on the mountain Puy de Dôme in Auvergne, with the result that the column of mercury there supported by the air was three inches shorter than near sea level.

Solubility of Gases in Liquids. Henry's[2] law (1805) states that *the mass of gas dissolved by a liquid, at any constant temperature, is proportional to the partial pressure of the gas in equilibrium with the liquid.* Qualitatively, this law is familiar to any one who has opened a bottle of a carbonated beverage: in the closed bottle, the partial pressure of carbon dioxide is high and the gas remains in solution; on exposure to the atmosphere, the partial pressure is reduced at once as a result of diffusion and the gas comes out of solution with effervescence. Quantitatively, solubility may be expressed in several ways. Perhaps the most logical unit of solubility is the mole fraction, or the ratio of the number of moles of dissolved substance to the total number of moles in the solution. In the case of gases in solution it is more usual to express their concentration in terms of volumes. The Ostwald[3] solubility coefficient is the ratio of the volume concentration of the gas in the liquid to that in the gaseous state, at the pressure and temperature of the experiment. The Bunsen[4] absorption coefficient is the volume of gas, reduced to 0° and 760 mm. Hg, which is dissolved by one volume of solvent at the experimental temperature when the partial pressure of the gas is 760 mm. Closely related to the latter unit is the solubility in volumes per cent, a unit widely used in physiological work. This means the number of volumes of gas at 0° and 760 mm. which can be dissolved by 100 volumes of the solvent under the experimental conditions. By applying the laws of Boyle, Charles, and Henry, the solubility in the latter units may

[2] William Henry (1774–1836) was a physician and manufacturing chemist in Manchester, England, and a friend of Dalton.

[3] Wilhelm Ostwald (1853–1932) was for many years Professor of Physical Chemistry at Leipzig. He was one of the founders of the *Zeitschrift für physikalische Chemie,* and indeed of the science of physical chemistry itself. He was famous as a teacher; his textbooks have been widely used, and most of the early chairs of physical chemistry, in this country as well as abroad, were occupied by his pupils.

[4] Robert Wilhelm Bunsen's (1811–1899) name is familiar to all from his invention of the laboratory gas burner (1859). Most of his long career as a teacher was spent at Heidelberg. He did important work in many branches of chemistry.

be reduced to Ostwald units. If the solubility of the gas accurately follows Henry's law, it may be seen that the solubility in Ostwald units is independent of the partial pressure of the gas in equilibrium with the solution.

The solubility of gases in general decreases with rise in temperature.

Surface Tension. The interface or phase boundary between a liquid and a gas is characterized not only by the continual evaporation and condensation of liquid which produce the equilibrium between the same substance in the two states, but also by the existence of unbalanced forces of attraction between the molecules. A particle in the interior of a pure liquid, being completely surrounded by similar particles, is under the influence of equal attractive forces in all directions. A particle of liquid in the surface layer, however, is acted upon by unbalanced forces, since the molecules of gas or vapor above it, being farther away, attract it much less than the liquid particles below it. Accordingly, if a particle is brought from the interior of the liquid into the surface, work must be done in overcoming the forces attracting it downwards. Hence the particles in the surface layer possess more potential energy than those in the liquid. Any increase in the area of the surface involves bringing more particles into the surface, which means that work is done in such a process. This work may be considered to be done against a force in the plane of the surface which opposes stretching of the surface. The surface tension is the amount of this force of contraction across a line of unit length in the surface, and it has the dimensions of force per unit length, or MT^{-2}. Surface tension is usually expressed in c.g.s. units, as dynes per centimeter.

Surface energy, which corresponds to the work done in producing an increase in the area of the surface, is equal to the surface tension multiplied by the increase in area, having the dimensions ML^2T^{-2}, which are those of work or energy. In the c.g.s. system surface energy is expressed in ergs (dynes $\times$ centimeters).

The existence of surface tension may be illustrated by simple qualitative experiments. If a soap film is formed on a wire ring

containing a loose loop of thread in its plane, the thread remains loose and may be pushed into any desired shape. If the film is broken inside the loop of thread, leaving the outer zone of film between the thread and the wire intact, the thread at once assumes the form of a circle. This indicates that the soap film behaves like a stretched membrane, its surface tension tending to make its area as small as possible.

The quantitative measurement of the surface tension at the interface between a pure liquid and a gas (usually air) is most easily made by the capillary rise method. If a narrow glass tube is held vertically and dipped into a liquid such as water, the liquid rises in the tube, reaching equilibrium at a level considerably above that of the main interface. This is a result of the fact that water wets glass. If the glass is absolutely clean, a thin film of water crawls upwards along the bore of the tube, making a water-air surface which tends to contract. At equilibrium the upward force, due to surface tension acting vertically, must be equal to the downward force, due to the action of gravity on the suspended column of water, or

$$2\pi rs = \pi r^2 hdg.$$

Here r is the radius of the tube, s the surface tension in dynes per centimeter, h the difference in level, d the density of the liquid, and g the value of the acceleration due to gravity, which is the number of dynes in a force of 1 gram. Hence the surface tension is given by the equation

$$s = \tfrac{1}{2} rhdg.$$

This equation implies that the angle of contact between water and glass is zero, or that the whole of the effective surface tension is directed vertically. The latter assumption seems to be strictly true for glass and many pure liquids, such as water or benzene. If the liquid does not wet the tube, which is true for mercury and glass, the meniscus is convex upwards and the liquid moves down in the tube below the main level. Here again, if the angle of contact is really zero, the same calculation holds.

A method which has been used for relative measurements of surface tension is the drop weight method. If a liquid is allowed

to fall slowly from a narrow orifice, each drop will break off when its weight just exceeds the force due to surface tension. A difficulty here is that the length along which the surface tension acts is not easily obtainable. The drop becomes constricted at the top before it breaks off, and a fraction of it is left hanging. If it broke off sharply in the plane of the surface from which it was hanging, the force up would be $2\pi rs$, where r is the radius of the upper plane surface of the drop. In general, however, the weight of the drop is not equal to this force because the plane of cleavage has a smaller radius. For relative measurements the method is used on the assumption that for two liquids

$$\frac{w_1}{w_2} = \frac{s_1}{s_2}$$

where w is in each case the weight of a single drop and s is the surface tension. The accuracy is increased by catching several drops and using an average weight.

A simplified but less accurate form of this relative method has been much used in biological work. A stalagmometer, which is simply a pipette with a special flattened tip, is filled with the liquid, and the number of drops formed from a given volume of liquid is counted. The weight of a drop is the density $\times$ the volume $\div$ the number of drops, or

$$\frac{s_1}{s_2} = \frac{d_1 n_2}{d_2 n_1}$$

if the volumes are identical and n is the number of drops formed. The method may be illustrated, in a crude qualitative way, by counting the drops of two liquids of widely different surface tensions, such as ether and water, which are delivered from an ordinary volumetric pipette.

Still another method has found favor with biological workers in recent years. This is the ring method, in which the force required to detach a platinum ring lying in the surface is measured directly by a balance such as a torsion or chain balance. The liquid forms a film over the ring, so that when the latter is lifted

the surface may break along a line of length $4\pi r$, if r is the radius of the ring. This method has been criticized on the ground that rings of different sizes give different results, presumably because the double film of liquid does not rise as a perfect cylinder of the radius of the ring. With a single ring, however, it seems to give true relative results if calibrated by a liquid of known surface tension. The method has been much used in following spontaneous changes in surface tension with time, such as occur at the surface of blood serum and other colloidal solutions. In ease and rapidity of execution it surpasses other methods.

Interfacial Tension. Surface tension exists not only at the interface between a liquid and a gas, but also at the interface between two immiscible liquids. This tension may be measured by suitable modifications of the capillary rise method, the drop method, or the ring method. A knowledge of such tensions is probably of more importance in physiology than that of air-liquid tensions, because of the existence of immiscible liquid phases in living cells.

Surface tension should theoretically exist at the interface between a solid and a liquid, but no method is known for its direct measurement, because of the rigid immobility of such an interface.

Viscosity. Both gases and liquids possess internal friction or viscosity, by which is meant the resistance exerted to a shearing stress, or to any force tending to make one layer of the fluid move faster than another adjacent layer. Qualitatively a liquid of high viscosity flows slowly through a tube and is poured or stirred with difficulty. A liquid of low viscosity has the opposite properties, and is also described as being mobile, or of high fluidity. The coefficient of viscosity (or simply the viscosity) of a fluid is defined as the tangential force required to move one face of a centimeter cube of the material with unit velocity (1 cm./sec.) past the opposite stationary face of the cube. The dimensions of viscosity are those of force $\times$ distance/(area $\times$ velocity), or pressure $\times$ time, or $ML^{-1}T^{-1}$.

The first thorough study of the viscosity of a liquid was made

by Poiseuille[5] (1843), who measured the flow of water through glass capillary tubes. He found that the rate varied directly as the pressure difference, directly as the fourth power of the radius of the tube, and inversely as the length of the tube. Poiseuille's law was later derived mathematically on the assumption that the liquid moved in layers of gradually increasing velocity as the center of the tube was approached, the layer of liquid molecules next to the wall of the tube being stationary. This law may be written

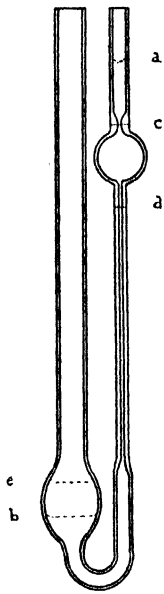

FIG. 2. Ostwald viscosimeter.

$$\frac{v}{t} = \frac{\pi r^4 p}{8 l \eta}$$

where v is the volume of liquid flowing in time t, under a pressure difference p, through a tube of radius r and length l. If all these quantities are expressed in c.g.s. units, the quantity η is the coefficient of viscosity, as defined above. Poiseuille's law holds very exactly for pure liquids if measurements are made with tubes of sufficiently small radius and of length above a certain minimum.

This law is used in the Ostwald viscosimeter (or viscometer), an instrument in which a fixed volume of liquid is allowed to flow by gravity through a capillary tube. The flow is started with the surfaces of the liquid at about a and b, Fig. 2. The quantity measured is the time required for the upper meniscus to fall from the mark at c to that at d. After this time the lower surface may be at e. The pressure head is gradually decreasing during the measurement, but if the same total volume of liquid is always used in filling the instrument, the pressures acting in the case of any two liquids must be proportional to their densities, since the distance between the upper and lower surfaces at any stage of the measurement is fixed by the shape of the in-

[5] Jean-Léon-Marie Poiseuille (1799–1869) was a French physician who did this important work in pure physics as a result of his interest in the rate of flow of blood in vessels of different sizes.

strument and the total volume. Accordingly relative viscosities are calculated from the formula

$$\frac{\eta_1}{\eta_2} = \frac{t_1 d_1}{t_2 d_2}$$

which follows from the application of Poiseuille's law to the measurement with each of two liquids. Here η represents coefficient of viscosity; t, time of flow between the marks; and d, density of the liquid. To get a value for the viscosity of any liquid, the instrument must be calibrated with a liquid of known viscosity. The viscosity of certain standard liquids has been accurately determined by measurements with tubes of known size and the application of Poiseuille's law. The figures may be found in tables such as the International Critical Tables.

The viscosity of liquids is quite sensitive to temperature changes; for accurate measurements the instrument used should be immersed in a constant temperature bath. In the case of water and aqueous solutions at ordinary temperatures, the viscosity decreases about 2 or 3 per cent for each Centigrade degree of rise in temperature.

The most obvious physiological application of viscosity is in the effect of narrow blood vessels on the rate of circulation of the blood. Much has also been written about the viscosity of muscles. Although for such semi-solid materials the coefficient of viscosity may not be accurately measurable, yet some conception allied to viscosity must be necessary for an adequate understanding of such changes in shape as those involved in muscular movements.

PROBLEMS

1. A volume of gas, collected over water, was found to be 47.25 cc. at 20°C. The barometer reading was 76.46 cm. Hg. At 20°C. the vapor pressure of water is given as 17.5 mm. Hg. What is the volume of this gas under standard conditions?

2. The solubility of oxygen in water at 20°C. is given as 0.004339 grams per 100 grams of water, the total pressure of the oxygen and water vapor in equilibrium with the solution being

760 mm. Hg. Calculate the value of this solubility in Bunsen units, in Ostwald units, and in volumes per cent.

3. The solubility of nitrogen in water at 20°C. is given as 0.001901 in the same units as in the preceding problem. Calculate the solubility of air in water at 20°C. and 1 atmosphere total pressure, assuming air to consist of 21 per cent by volume of oxygen and 79 per cent of nitrogen. Express the result in volumes per cent.

4. The interfacial tension between ether and water was determined at 25°C. by the capillary rise method. The radius of the tube was 0.355 mm. and the difference in levels was 21.3 mm. Calculate the value of the interfacial tension in dynes per centimeter, taking the density of water as 0.997 and that of ether as 0.710.

5. The c.g.s. unit of viscosity is known as the poise. The absolute viscosity of water at 20°C. is 1.009 centipoises, while that of a 20 per cent sucrose solution is 1.967. If the time of flow for water in a certain Ostwald viscosimeter at 20°C. is 80.2 seconds, what would be the time of flow for a 20 per cent sucrose solution in the same instrument? (Take the density of water as 0.997, and that of the sucrose solution as 1.081.)

6. Viscosities are sometimes expressed, not in absolute or c.g.s. units, but as relative viscosities, the standard for liquids being water at the temperature of the experiment. What is the relative viscosity of the sucrose solution of the preceding problem?

7. Viscosities are also sometimes expressed as fluidities, the fluidity of a liquid being defined as the reciprocal of its absolute viscosity in poises or c.g.s. units. Calculate the fluidities of the two liquids of the preceding problem.

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CHAPTER III

SOLUTIONS

Definitions. A solution has been defined as a one-phase system composed of two or more molecular species. This means that in a true solution there are no boundary surfaces between particles of the components of more than molecular size. A gas mixture such as air is a solution. Sand and water form a mechanical mixture; the boundary surfaces are obvious and the phases are readily separable by mechanical means. Sugar and water, salt and water, and alcohol and water form solutions; no boundary surfaces are to be detected. While the components can be separated, this usually involves a process such as distillation or freezing in which a new phase, vapor or solid, is produced. Theoretically there is no difference, other than their chemical individuality, between the components of a solution. The terms solvent and solute are used purely as a matter of convenience, the solvent being that component which is present in greater excess. The solutions which have been most studied are liquid solutions, especially those in which water is the solvent, and it is such aqueous solutions which are of most importance in physiology, owing to the universal preponderance of water in living organisms. There are, however, gaseous solutions, as has been mentioned, and solid or crystalline solutions, such as certain metallic alloys, and systems composed of two or more solid salts in which no phase boundaries can be detected. The following discussion will be limited to rather dilute aqueous solutions, owing to their predominant importance in physiology.

Units of Concentration or Composition. To a biological chemist a one per cent solution, say of salt in water, usually means a solution prepared by dissolving one gram of salt in enough water to bring the volume up to 100 ml.[1] This method of

[1] The abbreviation ml. means milliliter, one one-thousandth of a liter. The term cubic centimeter (cc.) is being replaced by ml., as the cc. is not exactly one one-thousandth of the liter, and the liter is the international standard of volume.

expressing composition may be convenient, but is not exact unless the temperature is specified. It is also illogical, as one per cent means one part in one hundred of the same units. A one per cent solution strictly should have one part by weight of solute in one hundred parts by weight of solution. In analytical chemical work the usual unit is the molar concentration (molarity) or equivalent concentration (normality). A molar solution is one containing 1 mole or gram-molecular weight of solute in 1 liter of solution at some specified temperature, usually 20°C. A normal solution contains one gram-equivalent weight of solute in 1 liter of solution at 20°. Since the value of the equivalent weight of a substance depends upon the reaction in which the substance is to take part, molar concentration is more definite than normality. Owing to the expansion and contraction of liquids with changes in temperature, the same solution at different temperatures usually has different molar concentrations or normalities. For this reason and others, physical chemists often express the composition of solutions in molal concentrations or molalities. A molal solution means one in which the components are present in the ratio of one mole of solute to 1000 grams of solvent. A given solution thus has the same molality at all temperatures. Molal concentrations can be translated into volume concentrations (molarity or normality) by making use of the density of the solution at the temperature to which the volume concentration is referred.

For theoretical purposes the most logical unit of composition is the mole fraction. This means the ratio of the number of moles of one component (e.g., a solute) to the total number of moles (solvent + solutes) in any given quantity of solution. The mole fraction of a solution is independent of temperature. It can be translated into molality if the molecular weights of the components are known, and into volume concentration if the density of the solution is also known. In the case of water it is customary to consider the molecular weight as that corresponding to the formula H_2O, 18.016, although liquid water probably consists of several types of associated molecules, such as $(H_2O)_2$ and $(H_2O)_3$, in equilibrium with some single molecules of H_2O.

As an example of the use of these various units, we may consider a solution containing 10 per cent by weight of cane sugar in water. The density of this solution at 20° is 1.038, or 1 ml. weighs 1.038 g. Therefore 100 ml. at 20° must contain 10.38 g. of sugar, or the volume percentage concentration is 10.38. The molar concentration is 103.8/342.2, or 0.3033 mole per liter at 20°. To get the molality it is necessary only to consider that 100 g. of solution contain 10 g. of sugar + 90 g. of water. Hence the amount of solution containing 1000 g. of water must contain 111.1 g. of sugar, or the molality is 111.1/342.2 or 0.3247. The mole fraction may be obtained from this figure by the relation

$$x = \frac{0.3247}{\frac{1000}{18.016} + 0.32} = \frac{0.3247}{55.51 + 0.32} = \frac{0.3247}{55.83} = 0.005816,$$

or from the weight percentage composition by the relation

$$x = \frac{\frac{10}{342.2}}{\frac{90}{18.016} + \frac{10}{342.2}} = \frac{0.02922}{4.996 + 0.029} = \frac{0.02922}{5.025} = 0.005815.$$

(These figures are identical except for the effect of rounding off the numbers to 4 digits.) The mole fraction of the solvent is $1 - x$, or 0.9942. The magnitude of these mole fractions indicates that a solution of concentration 0.3 M[2] is still very dilute when the whole possible range of mole fractions is considered. The mole fraction scale is particularly useful when it is desired to compare the properties of solutions varying widely in composition, such as may be obtained when the components are miscible in all proportions, as are alcohol and water. For solutions where the solubility is limited, as is the case with ordinary salts and water, the molality is usually the most convenient unit of concentration in physico-chemical work.

[2] The abbreviation M means molar, referring to the number of moles of solute per liter of solution at 20°C. Some writers use the same symbol for molal.

Vapor Pressure of Solutions. The vapor pressure of a solution is equal to the sum of the partial vapor pressures of its constituents, in agreement with Dalton's law of partial pressures. From a kinetic viewpoint it is to be expected that each constituent in the solution will have a lower vapor pressure than it would have in the absence of the other constituents. Fewer of its particles can cross the surface in any instant because of the presence of the particles of the other constituents.

In many cases the vapor pressure of a dissolved substance such as salt or sugar may be quite negligible in comparison with that of a solvent such as water. Here too the kinetic theory predicts that the solvent in the solution will have a lower vapor pressure than the pure solvent, and the quantitative dependence of this lowering of the vapor pressure on the composition of the solution is given by Raoult's[3] law (1887). This law applies exactly to very dilute or "perfect" solutions, and holds approximately (within 1 or 2 per cent) for solutions in which the mole fraction of the solute is as high as 0.02. According to Raoult's law *the fractional lowering of the vapor pressure of a solvent produced by the presence of a solute is equal to the mole fraction of the solute*, or

$$\frac{p_0 - p}{p_0} = x. \tag{1}$$

Here p_0 is the vapor pressure of the pure solvent, p is that of the solution, and x is the mole fraction of the solute. The vapor pressures must naturally be compared at the same temperature. An alternative statement of this law is

$$\frac{p}{p_0} = 1 - x \tag{2}$$

or *the ratio of the vapor pressure of the solvent in a dilute solution to that of the pure solvent is equal to the mole fraction of the solvent in the solution.* In this form the law is obviously a reasonable consequence of the kinetic considerations already mentioned.

[3] François Marie Raoult (1830–1901) was Professor of Chemistry at Grenoble, France. His work on vapor pressures and freezing points of solutions was done about 1880–1890, when he was 50 to 60 years old.

Boiling point. The boiling point of a liquid is defined as the temperature at which its vapor pressure is equal to the atmospheric pressure. The vapor pressure of any liquid increases with rise in temperature, and the effects of temperature on the vapor pressures of a pure solvent and of the solvent in a solution are approximately parallel (Fig. 3). Since at any given temperature

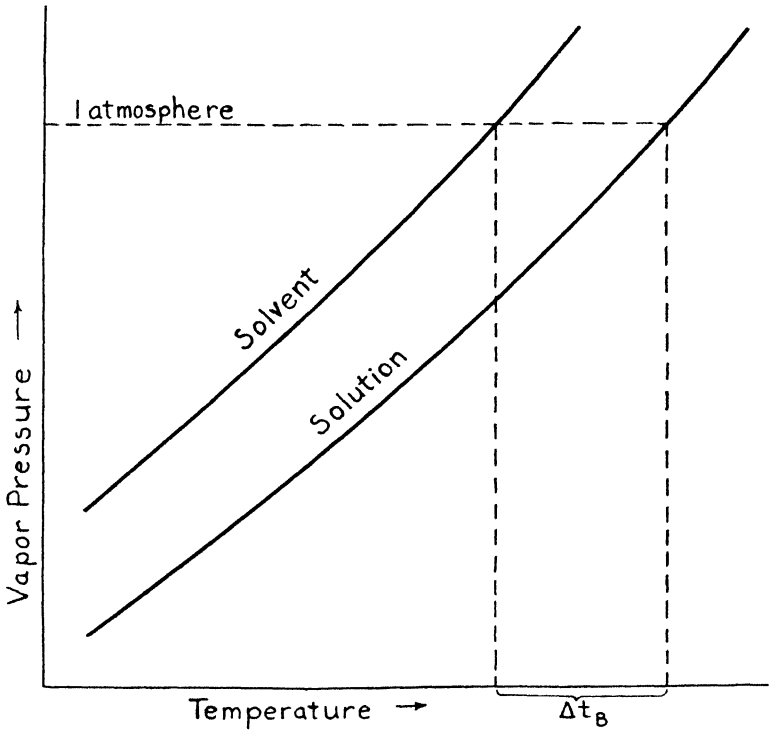

FIG. 3. Elevation of the boiling point.

the vapor pressure of a solution is below that of the pure solvent, it follows from this relation that the boiling point of a solution of a non-volatile solute is always higher than that of the pure solvent. By combining the mathematical relation between vapor pressure and temperature, which is known from thermodynamics, with Raoult's law, a relation has been derived between the elevation of the boiling point of a solution and its concentration. The simplest form of this relation is

$$\Delta t_B = k_B m \tag{3}$$

where Δt_B is the elevation of the boiling point, m is the molality of the solution (moles of solute per 1000 g. of solvent), and k_B is a constant of proportionality, the molal elevation of the boiling point. The value of k_B varies with the solvent; for water it is 0.515°C. This law holds approximately for dilute solutions of non-electrolytes. It has been much used in purely chemical work as a means of determining the molecular weights of substances in solution, but its biological application is limited by the destructive effect of boiling temperatures on biological substances in solution.

Freezing Point. The freezing point of a liquid is defined as the temperature at which the solid and liquid forms of the substance can exist together in equilibrium. At the freezing point the solid and liquid phases must have the same vapor pressure; if this were not so, the phase having the higher vapor pressure would distil over into the other, and the solid and liquid phases would not be in equilibrium. At the freezing point all three phases, solid, liquid, and vapor, must be in equilibrium together. This leads to the conception of the vapor pressure of a solid. At any given temperature a solid such as ice, for example, has a definite vapor pressure, and the latter increases with rise in temperature, but more steeply than that of the corresponding liquid (Fig. 4). This fact makes it necessary, as shown in Fig. 4, for the solution to have a lower freezing point than the pure solvent. Again thermodynamics gives quantitative expressions for the effect of temperature on the vapor pressures of the solid and liquid phases, and by combining these with Raoult's law an expression for the effect of concentration on the freezing point lowering of a solution can be obtained. In an approximate form applicable to dilute solutions, this may be written

$$\Delta t_F = k_F m \tag{4}$$

where Δt_F is the lowering of the freezing point, m the molality of the solute, and k_F a constant, the molal lowering of the freezing point. The value of k_F depends on the nature of the solvent, being 1.858°C. for water.

Equation (4) applies only to experiments in which the solid phase which separates is pure solvent, and is strictly exact only for very dilute solutions (up to 0.04 *M* for sucrose in water), giving results which may be in error by several per cent for solutions as concentrated as 1 or 2 *M*. It has been much used for molecular weight determinations in pure chemistry, as well as

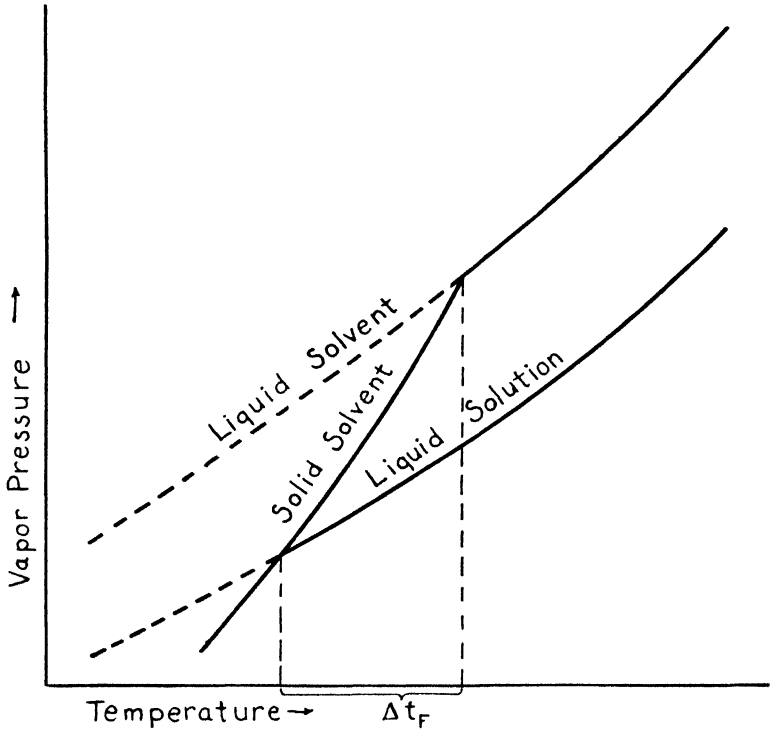

FIG. 4. Depression of the freezing point.

for studies of the concentrations of dissolved substances in biological liquids. Refinements of the freezing point method have made possible the use of this method in exact experimental studies of the behavior of dilute solutions, but here the calculations are much more complicated than is indicated by the simple equation given above.

Diffusion. The process of diffusion is not limited to gases. Molecules in a pure liquid or in a solution have considerable

freedom of movement. If a concentrated solution of a colored salt such as copper sulphate or potassium permanganate is put in the bottom of a tall glass jar, and a layer of water is carefully placed above the solution, the boundary between the colored solution and the water may be initially quite sharp. In the course of many days, if the jar is kept free from mechanical shocks or vibrations, it may be seen that the boundary slowly rises. Since the solution is heavier than water, this indicates that the water and solution are gradually being mixed together by forces working against gravity. Eventually the mixing becomes complete, and samples of the solution taken from any part of the jar will be found to have the same composition. Such diffusion is explained by the kinetic theory, as is the diffusion of gases. Each substance tends to move from places where it is more concentrated to places where it is less concentrated, so that eventually differences in concentration disappear. Graham's law has been shown to hold approximately for certain types of non-electrolytes in solution. For such substances which are chemically similar, a rough constancy is obtained by multiplying the diffusion constant by the square root of the molecular weight.

The dependence of the rate of diffusion on the concentration of the diffusing substance is given by Fick's[4] law (1855). According to this law, the small quantity of substance dS which passes through a given cross section in an infinitesimal time dt is given by the relation

$$dS = -Dq\frac{dc}{dx}dt. \qquad (5)$$

Here D is the diffusion coefficient, which is a constant depending on the nature of the substance and the solvent and on the temperature, q is the area of the cross section, c refers to concentration, and x to distance measured at right angles to the plane of the cross section. The symbol d means "the differential of"; that

[4] Adolf Fick (1829–1901) was Professor of Physiology at Würzburg. He is known for researches in muscle physiology and metabolism, as well as for this law of diffusion, which is really pure physics. At one time when his university was without a professor of physics, Fick is said to have delivered the lectures in that subject as well as in physiology.

is, a very small change in, or increment of, the quantity whose symbol follows it. The expression dc/dx therefore means the rate of change of concentration with respect to distance, or the concentration gradient. The minus sign implies that the substance diffuses in the direction of a negative concentration gradient, or towards the region of lower concentration. From the equation it follows that the physical meaning of the diffusion coefficient is that amount of substance which would diffuse in unit time and under unit concentration gradient across unit area, if the rate were constant during that time. The numerical value of D depends on the units in which the other quantities are expressed. In physico-chemical studies of diffusion it is usual to express quantity of substance in moles, concentration in moles per cc., distance in centimeters, area in square centimeters, and time in days. In these units D has the dimensions of square centimeters per day.

Fick's law was derived theoretically and has been verified by experiment with many dissolved substances.

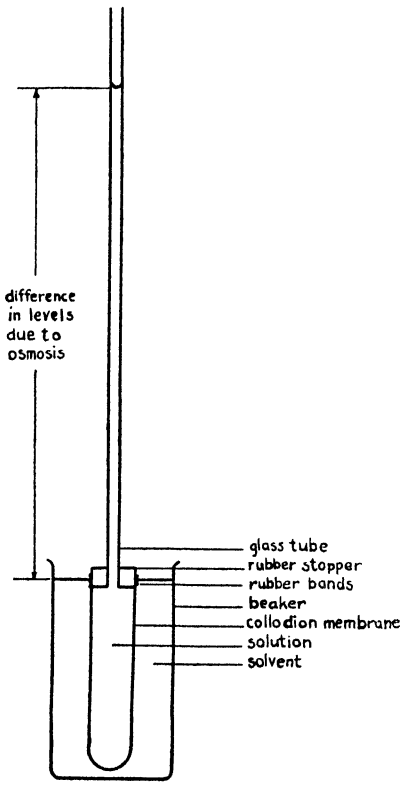

FIG. 5. Apparatus for demonstrating osmosis or measuring the osmotic pressure of a colloidal solution.

Osmosis. If two solutions having different concentrations of the same substance, or a solution and a pure solvent, are brought into contact, the theory of diffusion predicts that the solvent should tend to move from the region where it is more

concentrated (that is, from the dilute solution or the region of pure solvent) towards that where it is more dilute (that is, towards the more concentrated solution). The same result would be predicted if the two solutions were separated by a membrane permeable to the solvent, or even by a membrane permeable both to solvent and solute. This prediction is easily verified by experiment. For example, if a membrane of collodion or parchment paper, in the form of a sack or thimble, is filled with a 10 per cent sugar solution, fitted with a one-hole stopper bearing a vertical glass tube, and submerged in a beaker of distilled water, the level of the liquid in the glass tube is seen to rise rapidly (Fig. 5). Such *diffusion of a solvent through a membrane into a more concentrated solution is called osmosis.* In the example given, careful observation of the water outside of and below the membrane will show the presence of diffusion streaks, indicating that the dissolved sugar is also passing through the membrane, but in the opposite direction. The rise of liquid in the tube is only temporary; after a few hours the level falls to that of the outside water, and an analysis would show the sugar (and therefore the water) to be equally concentrated on both sides of the membrane. In this case the membrane is permeable both to water and sugar, but the osmosis is made evident at first because the smaller water molecules can diffuse faster. A sufficient explanation for osmosis is the tendency of the solvent to diffuse into the region where its concentration is lower.

Osmotic Pressure. In the experiment just described, the temporary difference in levels indicates a temporary difference in pressure between the two parts of the system. If a membrane is used which is absolutely impermeable to the solute but permeable to the solvent the pressure difference reaches a stationary limiting value. Such a membrane is described as semi-permeable. Semi-permeable membranes for aqueous sugar solutions have been prepared by precipitating copper ferrocyanide in the pores of porous clay cups, but their preparation is a matter of some difficulty. Membranes of collodion or parchment paper can be prepared which are semi-permeable to aqueous protein solutions. With a 10 per cent solution of egg albumin

in a collodion membrane, the difference in levels may be nearly a meter, and this difference may be constant for several days, or until the protein begins to be decomposed as a result of bacterial contamination or by hydrolysis.

The osmotic pressure of a solution is defined as equal to the hydrostatic pressure difference required to prevent further osmosis of solvent through a semi-permeable membrane into the solution. This excess pressure on the solution brings it into equilibrium with the solvent; the passage of solvent through the membrane does not cease altogether, but its rate is equal in both directions. The excess pressure has the effect of increasing the rate of diffusion of the solvent in the solution. A solution and a solvent may be brought into equilibrium across a semi-permeable membrane either by the spontaneous increase in pressure due to the rise of solution in a manometer tube as a result of osmosis, or by putting external pressure on the solution until osmotic flow appears to stop. In the former case the solution will be somewhat diluted by the entrance of solvent, so that the measured osmotic pressure is that of the solution as it is at the end of the experiment.

It is customary to speak of the osmotic pressure of a solution as a definite property like vapor pressure or concentration or freezing point. This is correct if the preceding experimental definition is always borne in mind. To say that a certain solution has an osmotic pressure of so many atmospheres does not mean that the solution, or any part of it, is at all times under such a pressure. It means simply that this pressure would be required to prevent osmosis of solvent into it through a semi-permeable membrane.

Osmotic Pressure and Vapor Pressure. Since the solvent and solution in an osmotic apparatus are in equilibrium as far as passage of solvent through the membrane is concerned, they must also be in equilibrium as regards passage of solvent through the vapor phase. If this were not so, a perpetual motion machine would be possible, which is contrary to the laws of thermodynamics. This equilibrium condition implies that the vapor pressure of the solvent in the solution has been increased

by the hydrostatic pressure imposed upon it so that it is now equal to that of the pure solvent at the lower pressure which exists outside of the membrane. (It will be recalled that normally, at the same total pressure, the vapor pressure of a solvent is lowered by the presence of a solute.) There is a thermodynamic equation for the effect of a change in total pressure on the vapor pressure of a pure liquid or a dilute solution. This equation is

$$P_1 - P_2 = \frac{RT}{V_0} \ln \frac{p_1}{p_2}. \tag{6}$$

Here P_1 and P_2 are total pressures, p_1 and p_2 are the corresponding vapor pressures, and V_0 is the volume occupied by 1 mole of the solvent. It is assumed in the derivation of this equation that V_0 is not changed by the presence of the solute, and that the vapor of the solvent follows the perfect gas law. The symbol ln means natural logarithm, or logarithm to the base e.[5]

In applying equation (6) to an osmotic pressure experiment, it is evident that the difference in total pressures is, by definition, equal to the osmotic pressure, or

$$\Pi = P_1 - P_2. \tag{7}$$

If the apparatus is set up as in Fig. 5, but with a truly semipermeable membrane, the lower pressure P_2 is evidently 1 atmosphere, and P_1 is the total pressure on the solution inside the membrane. Consider a portion of the solution before it is placed in the osmometer. The pressure being P_2, its vapor pressure is p_2. After osmotic equilibrium has been attained, its pressure is P_1 and its vapor pressure p_1. But p_1 is also, as was pointed out above, equal to the vapor pressure of the pure solvent at the total pressure P_2. Hence equation (6) may be rewritten

$$\Pi = \frac{RT}{V_0} \ln \frac{p_0}{p} \tag{8}$$

where p_0 is the vapor pressure of the pure solvent and p that of the solution, both under a total pressure of 1 atmosphere.

[5] The number e has the value 2.718+. A more useful figure to remember is the factor for converting natural logarithms to ordinary logarithms (base 10); the relation is $\ln x = 2.303 \log x$.

Equation (8) furnishes a rather exact means of calculating the osmotic pressure of a solution if its vapor pressure is known; it is found to hold within 1 or 2 per cent for aqueous sucrose solutions even up to 6 M[6].

Osmotic Pressure and Concentration. By combining equation (8) with equation (2) (Raoult's law), the following relation is obtained between the osmotic pressure of a solution and the mole fraction of the solute in it:

$$\Pi = -\frac{RT}{V_0} \ln (1 - x). \tag{9}$$

(It is to be remembered that x is the mole fraction of the solute, so that $1-x$ is that of the solvent.) This equation is known as the general osmotic pressure law, or the equation for the osmotic pressure of perfect or ideal solutions. Theoretically it ought to hold exactly for solutions in which there is no volume or heat change on mixing the solvent and solute, no association or dissociation of either solvent or solute, and no solvation or chemical combination between them. Its derivation assumes also that vapor pressure is a true measure of the escaping tendency of the solvent, and that its vapor obeys the perfect gas law. Experimentally, the most exact osmotic pressure measurements have been made with sugar solutions, and here not all of these conditions are satisfied.

For dilute solutions, equation (9) can be very considerably simplified. It can be shown mathematically that if x is a small fraction, $-\ln (1-x)$ is approximately equal to x. For this case equation (9) becomes

$$\Pi = \frac{RTx}{V_0}. \tag{10}$$

The mole fraction of the solute, x, is by definition equal to $N_A/(N_A+N_B)$, where N_A is the number of moles of solute in that portion of the solution which contains N_B moles of solvent. If N_A is small in comparison to N_B, x is approximately equal to the mole ratio N_A/N_B. The product $N_B V_0$ is the total volume of

[6] See Eucken, Jette, and LaMer (39): p. 213, Table 26b.

solvent in the portion of the solution under consideration this portion of solution is so chosen that it contains that v of solvent which weighs 1000 grams, then $N_B = 1000/$ $N_A = m$, where M is the molecular weight of the solver is the molality of the solute. Equation (10) then beco.

$$\Pi = \frac{RT\,mM}{1000\,V_0}.$$

If the solvent is water, $M = 18$ and $V_0 = 0.018$ liters, neglecting the difference between the volume of water at the experimental temperature and 4°C., at which the volume of the liter is defined. For dilute aqueous solutions the relation becomes simply

$$\Pi = RTm. \tag{12}$$

It is this equation which was found empirically by Morse to fit the results of his experiments on the osmotic pressure of sucrose solutions better than the earlier equation of van't Hoff (1885),[7] in which m is replaced by C, the concentration of the solute in moles per liter. The latter equation is simply

$$\Pi = RTC. \tag{13}$$

The van't Hoff equation (13) is formally identical with the perfect gas law (equation (8), Chapter 1), but attempts to follow up this resemblance as a means of explaining the mechanism of osmosis and osmotic pressure have led to considerable confusion. van't Hoff recognized that his equation could be expected to apply only to very dilute solutions; with this limitation, it has been extremely useful. Table I shows how equations (9), (12), and (13) differ from each other and from the values observed by Morse for the osmotic pressure of aqueous sucrose solutions at 20°C.

[7] Jacobus Hendricus van't Hoff (1852–1911), one of the founders of modern physical chemistry, was of Dutch birth, and taught at Utrecht and Amsterdam, later at Berlin. His name is well known to students of organic chemistry for his share in the development of the theory of space isomerism. His fame as a physical chemist rests not only on this osmotic pressure relation, and its extension to cover the osmotic properties of electrolytes, but also on his applications of thermodynamics to chemistry. He collaborated with Ostwald in founding and editing the *Zeitschrift fur physikalische Chemie.*

TABLE I
Osmotic Pressures of Sucrose Solutions at 20°C.

x	m	Π (obs.) atm.	Π (calc.) atm. $\Pi = RTC$	$\Pi = RTm$	$\Pi = -\frac{RT}{V_0} \ln (1-x)$
0.098	0.1	2.59	2.36	2.41	2.40
0.192	0.2	5.06	4.62	4.81	4.79
0.282	0.3	7.61	6.78	7.22	7.18
0 369	0.4	10.14	8.88	9 62	9 57
0.452	0.5	12.75	10.87	12 03	11.95
0.825	1.0	26.64	19.85	24.05	23 80

The experimental data in the first three columns were obtained by H. N. Morse and associates, as cited by A. Findlay: *Osmotic Pressure*, Longmans, Green and Co., 2nd ed., London, 1919, p. 42.

The table shows that the observed values are consistently higher than any of the calculated values. A better fit can be obtained by assuming a definite degree of hydration, such as $C_{12}H_{22}O_{11} \cdot 6H_2O$. The hydration number probably varies with the concentration, particularly at higher temperatures. The table further shows that distinctly better agreement is obtained by getting the calculated values according to Morse by equation (12) rather than according to van't Hoff by equation (13). It also shows that the formula for ideal solutions, equation (9), is so nearly identical with the Morse equation in the region of physiological importance, up to about 0.3 M, that the extra labor involved in the use of the logarithmic formula is quite unnecessary in biological work. Equation (12) is therefore recommended as a means of calculating the osmotic pressure of solutions of biological interest.

Osmotic Pressure and Temperature. All of the equations proposed for osmotic pressure indicate that it should be proportional to the absolute temperature. This proportionality has been fairly well confirmed by experiment. Frazer and Myrick (43) measured the osmotic pressure of concentrated sucrose solutions at 30°, and compared them with older measurements

of Berkeley and Hartley at 0°, re-calculating the latter to 30° on the assumption of proportionality to the absolute temperature. The maximum deviation between the observed and calculated values was 2.5 per cent, and in 3 cases out of 6 the deviation was well under 1 per cent. Theoretically this proportionality should not exist for solutions having an appreciable heat of dilution, but here the equations would have to be modified.

Colligative Properties of Solutions. The lowering of the vapor pressure, elevation of the boiling point, depression of the freezing point, and osmotic pressure are often called colligative properties of solutions because they are all thermodynamically related. If one of these properties is known for a given solution, any of the others can be calculated. While the most exact relations between them are somewhat more complicated, a very fair degree of accuracy may be obtained by the use of the approximate equations (1), (3), (4), and (12), which relate the values of these properties to the concentration of the solute. By eliminating the latter, equations may readily be obtained which connect the values of the four properties.

In the formulas relating the values of the colligative properties of solutions to concentration, there is no explicit statement as to the nature of the dissolved substance. Except for the important class of electrolytes, which is to be considered later, these equations apply to any solute. For example, Raoult's law relates the vapor pressure to the mole fraction of the solvent, irrespective of the nature of the solute which is effective in giving it this mole fraction. It follows that the equations should apply equally well to solutions containing several solutes, and this has been borne out by experiment. In the approximate relations containing molality, it is the sum of the molalities of all solutes which should be used; in equations containing the mole fraction of the solvent, the moles of all solutes must be counted in calculating the value of this fraction. Each of the colligative properties is therefore additive with respect to several solutes.

The Distribution Law for Dilute Solutions. The law of Henry, which was stated in Chapter II as applying to the solu-

bility of gases in liquids, is really of much wider application. A more general statement of this law is that the vapor pressure of the solute in any dilute solution is proportional to its mole fraction in the solution, or

$$p = kx. \tag{14}$$

If such a solution is shaken up with a second solvent, not miscible with the first, the solute will distribute itself between the two solvents, if it is at all soluble in the second. If the two phases or solutions are in equilibrium as a result of the shaking, they must also be in equilibrium through the vapor phase; that is, the solute in both solutions must have the same partial vapor pressure. By applying equation (14) to both solutions, we get $p_1 = k_1 x_1$ and $p_2 = k_2 x_2$. Since the vapor pressures are equal, it follows that

$$\frac{x_1}{x_2} = \frac{k_2}{k_1} = k_D, \tag{15}$$

where k_D is a new constant called the coefficient of distribution or partition. The value of k_D depends on the nature of the solvents and the solute as well as on the temperature. Equation (15) is the distribution law; it means that *when a solute is in distribution equilibrium between two immiscible solvents, the ratio of the mole fractions of the solute in the two solvents is a constant, independent of the total amount of either solvent or of the total amount of solute.* This law applies with considerable exactness to dilute solutions; apparent exceptions occur when the solute is associated in one solvent but not in the other. The law may also be stated in terms of molalities or concentrations, which are approximately proportional to the mole fractions in dilute solutions. Henry's law is a special case of the distribution law, being concerned with the distribution of a gas between a gaseous phase and a liquid solution.

Biological Applications of Osmotic Pressure. One of the first applications of the idea of osmotic pressure to living cells was made by de Vries[8] (1882) in his study of plasmolysis. This term

[8] Hugo de Vries (1848–), Professor of the Anatomy and Physiology of Plants at Utrecht, by these observations found a difference between electrolytes and non-electrolytes which was used by van't Hoff as confirmatory evidence for the theory of electrolytic dissociation of Arrhenius.

is applied to the apparent shrinkage of the cell contents of plant cells when they are placed in rather concentrated solutions. It is explained by assuming the existence of an elastic membrane around the protoplasm, the membrane being easily permeable to water but not to many solutes. This protoplasmic membrane is not to be confused with the visible cell wall, which is not elastic, but rigid, and freely permeable to dissolved substances. If the solution bathing the cells has a higher osmotic pressure than the cell contents, water tends to flow out of the cell, which therefore contracts and draws away from the wall. If the reverse is true, water tends to go in, but expansion is limited by the rigid cell wall. By testing cells with a series of concentrations of a given solute, it is possible to find a limiting concentration above which plasmolysis is just perceptible. The osmotic pressure of this solution is evidently close to that of the cell contents. In this way de Vries determined the concentrations of different substances which were isotonic with (having the same osmotic pressure as) the cell contents, and found that for non-electrolytes these concentrations were equimolar. The method became of use in pure chemistry in determining the molecular weight of raffinose, when that sugar had been newly discovered and little studied by other means.

If plant cells are put into hypotonic solutions (that is, solutions of lower osmotic pressure than the cell contents), the tendency towards osmotic swelling produces the stiffening of the plant structure known as turgor. This explains much of the rigidity of living green plants, and the wilting which occurs when the cells die and lose their semi-permeability. The idea of osmotic flow from a hypotonic solution has also been used to explain, in part at least, the rise of sap in trees.

In the case of animal cells, plasmolysis does not occur because there are no stiff cell walls. Osmosis may produce changes in the size of the cells, however, as is shown by the microscopic observation of red blood cells in solutions of varied concentration. Sufficiently dilute solutions cause the cells to burst when the swelling force exceeds the mechanical resistance of the cell membrane or other structure. This limit of fragility of red cells was

studied in 1883 by Hamburger[9] (51), who found that with cells of the same type and different non-electrolytes it was attained at the same molar concentration. Erythrocytes from different species of animals have different limits of fragility, and in no case is the solution producing hemolysis (bursting or stretching of red blood cells so that pigment escapes) isotonic with the cell contents.

The osmotic pressure of blood cells may be determined by finding that concentration of solute which causes no change in the size of the cells, provided that the cell is impermeable or very slowly permeable to the solute used. This is approximately true for most of the ordinary salts and sugars. The sizes of individual cells may be determined by measurement with a micrometer microscope, or the relative volume of a mass of cells may be found by the hematocrit method. This consists in centrifuging cell suspensions in narrow glass tubes of uniform bore. One difficulty is that very high speeds or long times of centrifuging are required to pack the cells down to constant volume. In such experiments it is of course necessary to have the same number of cells per unit volume of the suspension in each tube. By this method it has been found that the osmotic pressure of the erythrocytes of different mammals is remarkably constant, even though the limits of fragility of the cells vary with the species of animal. Mammalian red cells are approximately isotonic with 0.95 per cent NaCl, which is 0.16 *M*.

The freezing point method has been applied to estimate the osmotic pressure of various body fluids. For mammalian blood serum the freezing point is at about $-0.56°$C., which means that the molal concentration of a non-electrolyte of the same osmotic pressure would be $m = \Delta t_F / k_F = 0.56/1.86 = 0.30$. For NaCl and other simple salts, however, the solutions which freeze at the same temperature as the serum have a concentra-

[9] Hartog Jacob Hamburger (1859–1924) was a Dutch physiologist who taught at Utrecht and Groningen. The work mentioned above is said to have been the first application of physical chemistry to animal physiology. By this method he found a distinction between electrolytes and non-electrolytes which was used by van't Hoff as further evidence for the ionic theory.

tion of about 0.16 *M*. This is the same concentration which is isotonic with the cell contents; hence the red blood cells and the serum have the same osmotic pressure. This result was to be expected, since the cells are freely permeable to water, and their bi-concave shape indicates that they are not under any excess hydrostatic pressure.

In experimental physiology, tissues are usually bathed with salt solutions having the same osmotic pressure as the tissue fluids, so as to avoid possible changes or injuries due to osmosis. Such a physiological salt solution is sometimes referred to as "normal saline" or even "normal sodium chloride." The latter expression is unfortunate because of possible confusion with the special meaning given to the word normal by analytical chemists. "Physiological salt solution" is a preferable designation. Strictly no solution of sodium chloride *per se* is physiologically normal as far as living tissues are concerned, since a physiological salt solution should be balanced; that is, it should contain the ions of other salts in the naturally occurring ratios. Since the osmotic pressure of the blood of cold-blooded animals is less than that of mammals, the tissues of frogs and turtles are usually treated in physiological experiments with 0.65 per cent sodium chloride, which is 0.11 *M*.

PROBLEMS

1. Calculate the percentage composition, molality, and mole fraction of NaCl in a physiological salt solution containing 9.5 g. of NaCl per liter of solution at 20°C. Take the density of the solution as 1.0050 at 20°C.

2. The vapor pressure of water at 37°C. is 47.1 mm. Hg. Calculate the vapor pressure of the water in equilibrium with blood at this temperature, assuming the blood to be isotonic with a 0.30 molal sucrose solution.

3. Calculate the osmotic pressure and vapor pressure at 20°C. of an aqueous solution which freezes at −0.718°C.

4. If the molecular weight of sucrose is 342, and a 3.42 per cent sucrose solution is found by plasmolysis experiments to be

isotonic with a 5.96 per cent raffinose solution, what is the molecular weight of raffinose?

5. Explain what happens to wet strawberries which have been sprinkled with sugar and kept for several hours.

6. Suppose that a fatty substance is to be removed quantitatively from an aqueous solution by extraction with a fixed amount of ether. Apply the distribution law to show whether it will be more completely removed by a single extraction with all of the ether, or by several successive extractions with small portions of it.

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97. NOYES and SHERRILL, Chapter III.
127. WASHBURN,[10] Chapters XI–XIV.

[10] Edward Wight Washburn (1881–1934), late Chief Chemist of the Bureau of Standards, Washington, D.C., was formerly Professor of Physical Chemistry at the University of Illinois. He did important research work on the properties of solutions, and was editor-in-chief of the *International Critical Tables* (87). Shortly before his death he devised the first practical method of preparing heavy water, which contains the hydrogen isotope of atomic mass 2.

CHAPTER IV

Solutions of Electrolytes; The Law of Mass Action

Historical Development. The first volume of the *Zeitschrift für physikalische Chemie* (1887) contains two important papers by van't Hoff (122) and Arrhenius[1] (5) which form the basis for much of modern physical chemistry. While these papers were not the first publications in which the authors expressed their new views on the theory of dilute solutions, they were effective in bringing the new ideas before a wider circle of scientists. These papers have recently been reprinted in English (124) and still form a fascinating approach to the subject.

van't Hoff began by using the data of Pfeffer[2] (1877) on the osmotic pressure of cane sugar solutions to show that the osmotic pressure followed Boyle's law in being directly proportional to the concentration. The data of de Vries on plasmolysis were shown to agree with this law. van't Hoff showed further that Pfeffer's data at various temperatures justified an extension of the law of Gay-Lussac or Charles to osmotic pressure. Additional support was found in some work of Hamburger, who had shown that red blood cells were isotonic with the same solution at 0° and at 34°. Still using Pfeffer's data, he then justified the extension of Avogadro's law to solutions, which brought out the quantitative identity between the laws of gas pressure and of osmotic pressure for dilute solutions. Further evidence was found in data of Raoult on vapor pressures and freezing points of solutions, which verified van't Hoff's deduction that isotonic

[1] Svante August Arrhenius (1859–1927) was for much of his long scientific career the Director of the Laboratory of Physical Chemistry of the Nobel Institute in Stockholm. His fame of course rests largely on his theory of electrolytic dissociation. Students of biology will find many interesting applications of physical chemistry in his *Immunochemistry* (6) and his *Quantitative Laws in Biological Chemistry* (7).

[2] Wilhelm Friedrich Philipp Pfeffer (1845–1920) was a professor of botany who taught in several German universities, his last post being at Leipzig. He is regarded as one of the founders of the science of plant physiology, while his name is familiar to every student of chemistry because of these early measurements of osmotic pressure.

solutions should have the same vapor pressure and freezing point. For solutions of many of the ordinary acids, bases, and salts, the data did not fit the simple formula $\Pi = RTC$; van't Hoff made the formula fit the data by introducing a factor i which is now usually called the van't Hoff factor. For these substances the relation proposed was $\Pi = iRTC$. He calculated values of i from freezing point data, and showed that the same values, when applied to the earlier studies of Guldberg and Waage on chemical equilibrium, brought many apparent exceptions into agreement with the law of mass action of these authors. He pointed out that a value of i greater than 1 seemed to imply the existence of more molecules or osmotically effective particles than were indicated by the chemical formulas of the substances in question.

The paper of Arrhenius supplemented that of van't Hoff by showing a connection between the factor i and another property of solutions, their electrical conductivity. For non-conductors, i was 1. For many acids, bases and salts i was greater than 1 but less than 2. Arrhenius noticed that solutions of these substances were good conductors of electricity. By analogy with the established idea of the dissociation of the molecules of certain gases at high temperatures into two smaller molecules, he assumed that the solute molecules in a conducting solution were dissociated into smaller particles which he assumed to carry electric charges, thus accounting for the conductivity. He assumed this dissociation to be only partial at finite concentrations, but complete at infinite dilution. The extent of the dissociation was measured by the ratio of the conductivity (per gram molecule) at any given dilution to that at infinite dilution. He deduced a relation between the degree of dissociation, α (which he first called the activity coefficient), and van't Hoff's factor i. If n is the number of active or dissociated molecules, m the number of inactive or undissociated molecules, and k the total number of charged particles or ions into which each active molecule is dissociated, then, since i refers to the ratio of the total number of particles, ions and molecules, to the number of molecules indicated by the formula of the substance,

$$i = \frac{m + kn}{m + n}.$$

But since

$$\alpha = \frac{n}{m + n}$$

it follows that $i = 1 + (k - 1)\alpha$. He calculated values of α from conductivity measurements, and from these he calculated values of i which he compared with those obtained by van't Hoff from freezing point data. The approximate agreement of the two sets of values for i was taken as quantitative evidence for the validity of the new theory. Although according to modern ideas this agreement is believed to be largely a coincidence, yet it was instrumental in bringing about the acceptance of the theory.

This theory of Arrhenius, in spite of later modifications, is still one of the most important generalizations of physical chemistry. Its importance in physiology was early brought out by the fact that the work of such biologists as Pfeffer, de Vries and Hamburger was instrumental in providing evidence for it.

Electrolytes and Non-Electrolytes. Electrical conductivity or conductance is defined as the reciprocal of electrical resistance, the unit of measurement being the reciprocal ohm, sometimes called the mho. The specific conductivity of a solution is the conductivity of a centimeter cube of the solution. It is the reciprocal of the resistance of that amount of solution which is contained between two parallel flat electrodes 1 centimeter apart and of 1 square centimeter area on a side. Since the laws connecting electrical resistance with the size and shape of the conducting medium are known, it is not necessary for the electrodes used in practical work to have these dimensions. The resistance of a body varies directly as its length and inversely as the area of its cross section. Practically a conductivity cell is calibrated by making measurements with a solution of known conductivity, usually aqueous potassium chloride solution. Since conductivity is considerably influenced by temperature, the use of a constant temperature bath is necessary in making such measurements.

Arrhenius pointed out that all solutions could be divided into two classes, those which conducted the current readily, the electrolytes, and those which had an extremely high resistance or low conductivity, the non-electrolytes. The latter class includes most organic compounds with the exception of acids and bases. The electrolytes comprise the acids, bases, and salts. Electrolytes are further divided into strong and weak electrolytes. Here the division is not absolutely sharp, but in general the strong electrolytes, whose solutions have very high conductivities, include practically all salts and most of the common inorganic acids and bases, such as HCl, HNO_3, H_2SO_4, NaOH, KOH, $Ba(OH)_2$, $Ca(OH)_2$, etc. Examples of weak electrolytes are most organic acids and bases, as well as such inorganic substances as H_2CO_3, H_3PO_4, NH_4OH, H_3BO_3, H_2S. The laws governing the relation of conductivity to concentration for these two classes of solutions are quite different.

Strong Electrolytes. Aqueous solutions which are very good conductors of electricity have, at any given concentration, abnormally high values for the osmotic pressure and other colligative properties. For uni-univalent salts, the observed values of these properties may be almost twice as great as those calculated from the formulas given in the preceding chapter; that is, van't Hoff's factor i is nearly 2. This is explained by assuming that such electrolytes are completely dissociated into ions, each ion in the solution having the same osmotic effect as a molecule of a non-electrolyte. In fact, modern work has given evidence that, in the case of a strong electrolyte like NaCl, a crystal of the dry solid salt is made up of charged atoms or ions instead of molecules. When such a salt is dissolved in water, the ions may be hydrated or combined with some of the solvent, but they retain their charges and remain separate from one another. Most probably there are no undissociated molecules of a strong electrolyte. Why, then, do not solutions of sodium chloride, for example, have exactly twice the values for the colligative properties of solutions of a non-electrolyte like sugar, at corresponding concentrations? This question has puzzled physical chemists for many years. It is believed that an answer has been found in the

theory of Debye[3] and Hückel (1923). According to this theory of inter-ionic attraction, the strong electrolytes are completely dissociated, but the properties of their solutions are influenced by the electrostatic forces between the ions. From such purely physical considerations it has been possible for the first time to calculate coefficients which predict the values of the colligative properties as well as the conductivity of such solutions, and so far the agreement between calculation and experiment has been such as to justify the wide acceptance of the theory. This theory has now caused physical chemists to give up, for strong electrolytes, Arrhenius's idea of partial dissociation. The existence of the ions is not doubted, but for this class of substances the degree of ionization is believed to be 100 per cent. The quantity α, calculated from the conductivity ratio of Arrhenius, is not a measure of the degree of dissociation, and there is no simple relation connecting it with the concentration in the case of strong electrolytes.

Weak Electrolytes. The study of this interesting class of substances by Ostwald was largely instrumental in convincing scientists of the validity of the ionic theory, and it is to this class that the theory is still believed to apply almost in its original form. Solutions of weak electrolytes have values for the colligative properties only slightly greater than do non-electrolytes. They have, however, a definite and readily measurable conductivity; the explanation of the variation of their conductivity with concentration was one of the first quantitative achievements of the Arrhenius theory.

The equivalent conductivity of an electrolyte is defined as the specific conductivity of the solution divided by the number of gram-equivalents of solute contained in 1 cubic centimeter. It may be pictured as the conductivity which would be measured between parallel electrodes one centimeter apart but big enough to form the ends of a rectangular vessel holding that volume of the solution which contains one electrochemical equivalent in

[3] Peter Debye (1884-), of the University of Leipzig, is known as one of the leading physicists of the present day. He has previously held university positions in Munich, Utrecht, Zürich, and Princeton.

grams of the solute. If L represents the specific conductivity, C the concentration in equivalents (not moles, except for uni-univalent solutes) per liter, and Λ the equivalent conductivity as defined above, the relation between these quantities is

$$\Lambda = \frac{1000\, L}{C} . \qquad (1)$$

While the specific conductivity of an electrolytic solution decreases with the concentration, the equivalent conductivity

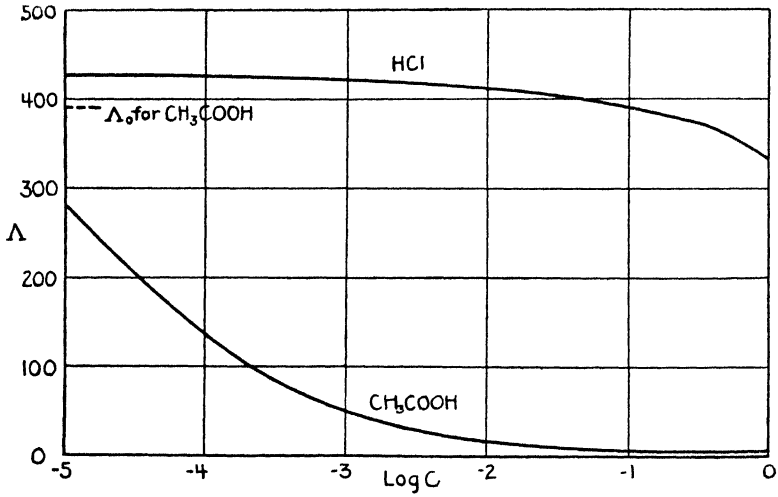

FIG. 6. Equivalent conductivities of weak and strong electrolytes.

increases as concentration is decreased. The difference between the courses of this change in equivalent conductivity with concentration for weak and strong electrolytes is shown in Fig. 6. In the case of a strong electrolyte like hydrochloric acid, the changes in Λ with C are relatively small, and Λ can be extrapolated to zero concentration with considerable certainty. For a weak electrolyte like acetic acid, the changes in Λ with C at low concentrations are tremendous, and Λ can be extrapolated to zero concentration only indirectly. For weak electrolytes, values of this extrapolated quantity, Λ_0, are obtained by the use of Kohlrausch's[4] law of the independent migration of ions

[4] Friedrich Wilhelm Georg Kohlrausch (1840–1910) was Professor of Physics at several German universities, and later head of the Physikalisch-

(1875). According to this law the equivalent conductivity of any electrolyte at zero concentration is the sum of two independent numbers, the equivalent conductivities of its ions, and, since at zero concentration the electrolyte is present wholly as ions, the conductivity of each ion under these conditions is fixed and independent of the other ions present. For example, Λ_0 for acetic acid may be obtained by the proper addition or subtraction of the Λ_0 values for sodium acetate, sodium chloride, and hydrochloric acid, the latter three quantities being obtainable by direct extrapolation.

According to the original theory of Arrhenius, the fraction of an electrolyte in the ionic form, or the degree of ionization, should be given simply by the ratio of the equivalent conductivity at the concentration in question to that at zero concentration, or

$$\alpha = \frac{\Lambda}{\Lambda_0}. \qquad (2)$$

For strong electrolytes this relation is no longer believed to be true, as has been said, but for weak electrolytes it is still used, being at least a very good approximation. The values of α so obtained for weak electrolytes have been found to vary with the concentration in quantitative agreement with a relation known as the Ostwald dilution law, which is an application of the law of mass action.

The Law of Mass Action. In 1867 Guldberg and Waage,[5] as a result of a study of reversible chemical reactions, made the

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Technische Reichsanstalt at Charlottenburg, near Berlin. He and his coworkers made very accurate measurements of the conductivity of solutions of electrolytes, some of which are still used for the standardization of conductivity cells. The book *Das Leitvermögen der Elektrolyte*, by Kohlrausch and Holborn, was for many years the standard work on the subject of electrolytic conductivity.

[5] Cato Maximilian Guldberg (1836–1902) was Professor of Applied Mathematics at the University of Christiania (the present Oslo), and a member of the directorate of the Norwegian railways. He was a brother-in-law of Peter Waage (1833–1900), who was Professor of Chemistry in the same institution. Their collaboration in studies of chemical affinity resulted in the important generalization which has made their names internationally known.

generalization that *the rate of any chemical reaction is proportional to the product of the active masses of the reacting substances.* For simple cases the active mass may be considered identical with the concentration. If more than one molecule of a substance takes part in the reaction, as shown by the chemical equation, the concentration of that substance must be raised to a corresponding power in the rate equation. This is the simplest form of the law of mass action, which applies equally well to gas reactions or to reactions in dilute solution, and for reversible reactions it applies to the rates of the reactions in both directions. In general for any such reaction of the type

$$m\,\mathrm{A} + n\mathrm{B} \rightleftharpoons p\mathrm{C} + q\mathrm{D},$$

the rate of the combination of the substances A and B is given by the expression

$$r_1 = k_1(\mathrm{A})^m(\mathrm{B})^n,$$

while the rate of the reverse reaction is

$$r_2 = k_2(\mathrm{C})^p(\mathrm{D})^q.$$

The parentheses mean concentrations of the substances whose symbols are enclosed. The constants of proportionality k_1 and k_2 are called the velocity constants of the two reactions; their values depend on the temperature and other environmental conditions, but not, for low concentrations, on the initial concentrations of the reacting substances. Such a reacting mixture, if left to itself, reaches a state of equilibrium in which no further reaction seems to be going on. Actually it is believed, in the case of chemical equilibrium, just as in the equilibrium between a liquid and its vapor, that molecular motion causes a continual interchange between the reacting molecules. The reaction does not stop, but the rates of the two opposing reactions are equal. This leads to a form of the law of mass action applicable to the equilibrium state, namely

$$\frac{(\mathrm{C})^p(\mathrm{D})^q}{(\mathrm{A})^m(\mathrm{B})^n} = \frac{k_1}{k_2} = K. \tag{3}$$

The constant K, the ratio of the two velocity constants, is called the equilibrium constant of the reaction. It is conventional to

write in the numerator of this mass action expression the concentrations of those substances which appear on the right hand side of the chemical equation, but of course the number obtained by writing the expression the other way up must be equally constant.

More recent work has shown that in concentrated solutions, or gases under high pressure, the concentration in moles per liter is not always a true measure of the active mass. Hence a new quantity, the activity, has been defined in such a way that when activity is substituted for concentration in a mass action equation the equilibrium constant is a true constant independent of concentration. For dilute solutions, or gases at low pressure, activity and concentration are identical.

Weak Electrolytes and the Law of Mass Action. If the ionization of a weak electrolyte is considered as a reversible chemical reaction, that of a weak acid, for example, may be represented as

$$HA \rightleftharpoons H^+ + A^-.$$

Applying the law of mass action gives the expression

$$\frac{(H^+)(A^-)}{(HA)} = K_a. \tag{4}$$

For the special case of a weak acid the equilibrium constant defined by this equation is called the ionization constant of the acid. If the solution contains only the weak acid and water, then

$$(H^+) = (A^-), \tag{5}$$

$$C = (H^+) + (HA) = (A^-) + (HA), \tag{6}$$

and the concentrations may be expressed in terms of the degree of ionization, α, by means of the following relations which follow from the definition of α:

$$\alpha = \frac{(H^+)}{(H^+) + (HA)} = \frac{(A^-)}{(A^-) + (HA)} \tag{7}$$

$$1 - \alpha = \frac{(HA)}{(H^+) + (HA)} = \frac{(HA)}{(A^-) + (HA)}. \tag{8}$$

By combining equations (4–8), one obtains

$$\frac{\alpha^2 C}{1 - \alpha} = K_a \tag{9}$$

or

$$\frac{\alpha^2}{(1 - \alpha)V} = K_a \tag{10}$$

where V is the reciprocal of the concentration, or the number of liters of solution containing one mole of the weak acid. The quantity V is sometimes called the dilution, and equation (9) or (10) is often called the Ostwald dilution law. Equations analogous to (4–10) may be readily deduced for the ionization of a weak base, where the chemical reaction would be

$$BOH \rightleftharpoons B^+ + OH^-$$

and the other equations would differ in having (B^+) in place of (A^-), (OH^-) in place of (H^+), and the ionization constant of the weak base, K_b, in place of K_a.

In order to submit the Ostwald dilution law to an experimental test, it was necessary to make use of equation (2), which relates α to conductivity measurements. Ostwald did this for a large number of weak acids and bases, combining equation (2) with (9) or (10) and calculating values of K_a and K_b. These were found to show a very satisfactory constancy for measurements with various concentrations, provided that the electrolyte was weak enough and the concentration low enough so that the concentration of any ion was not much over 0.001 M. An application of modern theories has made it possible to recalculate conductivity data in terms of ion activities, a process which gives constant values for K_a for higher concentrations and for somewhat stronger or "intermediate" electrolytes which did not appear to follow the Ostwald dilution law in its simple form (107, 78).

While the Arrhenius theory of partial dissociation and the law of mass action, as combined in the Ostwald dilution law, may be applied with considerable confidence to solutions of weak electrolytes, it is to be emphasized that they do not apply at all in the case of strong electrolytes. There is no such thing

as a dissociation constant for hydrochloric acid, sodium hydroxide, or sodium chloride. If conductivity data for such substances are put into the Ostwald dilution formula, values for K are obtained which are very far from constant. This anomaly of strong electrolytes, as it has been called, had baffled physical chemists for nearly forty years before it was explained by the theory of Debye and Hückel. Since the equations of this theory are somewhat complex, they will not be given here. Although a student may not be able to use this new theory, he should be warned against the indiscriminate application of the older theory to cases not covered by it.

Water as an Electrolyte. Water is a poor conductor of electricity, and the more carefully the water is purified, the lower its conductivity is. However, it can be measured, and Kohlrausch found the specific conductivity to be 4×10^{-8} reciprocal ohms at 18° C. From this measurement the equivalent conductivity of water may be calculated, and by the indirect method based on Kohlrausch's law it is possible to get a value for this quantity at zero concentration or infinite dilution, since it is the sum of the limiting values for H^+ and OH^-. In this way it was found that the concentration of either H^+ or OH^- in pure water was about 1.0×10^{-7} M. By applying the law of mass action to the reaction

$$H_2O \rightleftharpoons H^+ + OH^-,$$

it follows that the ionization constant of water would be

$$\frac{(H^+)(OH^-)}{(H_2O)} = \frac{1.0 \times 10^{-14}}{(H_2O)} = K.$$

Since in pure water and in ordinary dilute solutions the molar concentration of water is constant, the numerator of this mass action expression, the ion product (H^+) (OH^-), must also be constant. This ion product is usually represented by K_w, and it has the value

$$K_w = (H^+)(OH^-) = 1.0 \times 10^{-14} \qquad (11)$$

at 25°C. Its variation with temperature has been determined. A knowledge of its value makes possible a calculation of the con-

centration of hydroxyl ions in any solution when the concentration of hydrogen ions is known, and the reverse. It is to be emphasized that although the value of K_w was originally obtained from measurements of the conductivity of pure water, this value is constant, as has been shown by other methods, not only for pure water but for all dilute solutions of acids, bases, salts, or non-electrolytes.

Hydrolysis of Salts. One consequence of the slight ionization of water is that a salt of a weak acid or of a weak base does not form neutral solutions in water. The former case may be illustrated by considering a solution of sodium acetate of concentration C. Its hydrolysis consists in the reaction

$$NaA + H_2O \rightleftharpoons HA + NaOH$$

or

$$A^- + H_2O \rightleftharpoons HA + OH^-,$$

since the strong electrolytes NaA and NaOH are to be regarded as completely ionized. The extent or degree of hydrolysis is measured by the fraction x of the total salt which becomes unionized acid. From the chemical equation,

$$(HA) = (OH^-),$$

and hence

$$x = \frac{(OH^-)}{C}.$$

Since the ionic equilibrium for water must be satisfied,

$$(OH^-) = \frac{K_w}{(H^+)}$$

and

$$x = \frac{K_w}{C(H^+)}. \tag{12}$$

Since the ionic equilibrium for the weak electrolyte acetic acid must also be satisfied,

$$(H^+) = \frac{K_a(HA)}{(A^-)} = \frac{K_aCx}{C(1 - x)}. \tag{13}$$

The elimination of (H^+) from equations (12) and (13) gives

$$\frac{x^2C}{1-x} = \frac{K_w}{K_a},$$

which relates the degree of hydrolysis to the total concentration, the ion product of water, and the ionization constant of the weak acid. The ratio K_w/K_a is sometimes written as a single constant and called the hydrolysis constant of the salt. If the extent of hydrolysis is a small fraction, as it usually is, it may be neglected in the subtraction from 1 in the denominator, and the equation may be simplified to read

$$x = \sqrt{\frac{K_w}{CK_a}}. \tag{15}$$

For a 0.1 M solution of sodium acetate, $C = 0.1$, $K_w = 1.0 \times 10^{-14}$, and $K_a = 1.8 \times 10^{-5}$. Equation (15) gives $x = 7.5 \times 10^{-5}$ and equations (12) and (11) give $(OH^-) = 7.5 \times 10^{-6}$ and $(H^+) = 1.3 \times 10^{-9}$. In this case, although the fraction of the salt which is hydrolyzed is only 0.0075 per cent, yet the alkalinity or hydroxyl ion concentration is 75 times that of pure water.

Such a simple calculation of the hydrogen or hydroxyl ion concentration in a solution of a hydrolyzable salt does not apply to such a salt as sodium di-hydrogen phosphate or di-sodium hydrogen phosphate. Here the several ionization constants of phosphoric acid must be taken into account.

A salt of a weak base with a strong acid forms acid solutions in water, and the extent of its hydrolysis and the hydrogen ion concentration or acidity of the solution may be calculated by a similar procedure if the total salt concentration and the ionization constant of the weak base are known. Similar reasoning yields equations for salts of multivalent weak acids or bases, as well as for salts whose hydrolysis occurs not because the acid or base is poorly ionized but because it is slightly soluble. In such cases it is necessary to know the solubility product constant of the slightly soluble acid or base.

Solubility Product. According to the solubility product principle, *in a saturated solution of a strong electrolyte of low solubility the product of the concentrations of its ions is a constant, the con-*

centration of each ion being raised to a power equal to its subscript in the chemical formula. This rule applies to such substances as the precipitates of analytical chemistry; for example, to silver chloride, barium sulphate, ferric hydroxide. For an electrolyte of the type B_pA_q, the rule takes the form

$$(B)^p(A)^q = K_s. \tag{16}$$

The constant K_s is known as the solubility product; it varies with temperature, but is largely independent of the concentration of ions in the solution. If the rule is stated in terms of activities instead of concentrations, it may be derived by thermodynamics, and hence must be exact. Practically it may be regarded as an empirical rule which holds approximately for ion concentrations. It has received physiological application in attempts to explain the deposition of calcium salts to form bones in the animal body.

Common Ion Effect. This term is applied to two different things. It may refer to the decrease in solubility of a slightly soluble electrolyte produced by adding another electrolyte having an ion in common with the first. In this case the result can be calculated from the solubility product rule. For example, silver chloride is less soluble in dilute hydrochloric acid than in water. There are, however, exceptions, such as the re-dissolving of precipitated silver cyanide by an excess of potassium cyanide. This is explained as due to the formation of a feebly ionized complex ion, $Ag(CN)_2^-$. Other exceptions occur in cases where the solubility of a precipitate is increased instead of being decreased; for example, silver chloride is much more soluble in concentrated solutions of other chlorides than it is in water.

Another type of common ion effect is the repression of the ionization of a weak electrolyte by the addition of a strong electrolyte having an ion in common with it. The extent of this effect may be calculated by applying the law of mass action to the ionization of the weak electrolyte. Qualitatively this effect may be demonstrated by adding a solution of sodium acetate to a dilute solution of acetic acid containing the indicator methyl red; the color change indicates that the added acetate

ion removes hydrogen ion from the solution. A similar experiment may be done with ammonium hydroxide and ammonium chloride, using phenolphthalein as indicator.

Amphoteric Electrolytes. This class (also called ampholytes) consists of those electrolytes which may behave either as acids or bases, being capable of forming either hydrogen ion or hydroxyl ion in aqueous solution. Examples are aluminum hydroxide, zinc hydroxide, and the amino acids. The latter are weak electrolytes, and the law of mass action applies both to their ionization as acids according to the scheme

$$HROH \rightleftharpoons H^+ + ROH^-$$

and their ionization as bases, as

$$HROH \rightleftharpoons HR^+ + OH^-.$$

Application of the mass law to these two reactions gives the expressions

$$\frac{(H^+)(ROH^-)}{(HROH)} = K_a \qquad (17)$$

and

$$\frac{(HR^+)(OH^-)}{(HROH)} = K_b. \qquad (18)$$

In general K_a and K_b are not equal, and accordingly if such a substance is dissolved in pure water, the resulting solution will have unequal concentrations of H^+ and OH^-. This implies that (ROH^-) and (HR^+) must also be unequal, and a preponderance of either the negative or positive ion of the amphoteric substance may be demonstrated by its migration in an electric field. By the addition of acid or base it is possible to repress the ionization of one or the other form until a condition is reached in which the ampholyte shows no migration in an electric field, or migrates equally in both directions. This condition is called the isoelectric condition or isoelectric point of the ampholyte. The value of (H^+) at the isoelectric point is related to the ionization constants of the ampholyte as follows. If equation (17) is divided by equation (18), the expression (HROH) cancels out, and the same is true of (ROH^-) and (HR^+) for the condition

that the substance is at its isoelectric point. This gives

$$\frac{(H^+)}{(OH^-)} = \frac{K_a}{K_b}$$

as the relation defining (H^+) at the isoelectric point. Making use of equation (11), this becomes

$$(H^+) = \sqrt{\frac{K_a K_w}{K_b}}. \qquad (19)$$

This equation is true only for (H^+) at the isoelectric point, and strictly only for a uni-univalent ampholyte. For multivalent substances, however, the different ionization constants are usually so far apart that the location of the isoelectric point is practically fixed by the values of the largest K_a and the largest K_b.

According to a theory proposed by Adams (4) and Bjerrum[6] (17), the undissociated molecules of most ampholytes should be pictured rather as doubly-charged ions, with a positive charge at one part of the molecule and a negative charge at another. Such ions have been termed in German *Zwitterionen*, and they are referred to in English as zwitter ions, hybrid ions, or amphions. If this theory is adopted, the natural amino acids at their isoelectric points should be considered to have formulas of the type $^+H_3NRCOO^-$ instead of $H_2NRCOOH$, although both forms may be present. Combination with H^+ in acid solutions would then take place at the carboxyl group instead of the amino group, and combination with OH^- in alkaline solutions at the amino group instead of the carboxyl. This conception gives a new set of dissociation constants for each ampholyte, but they are so related to the old ones that equation (19) holds with either notation.

The theory of amphoteric electrolytes is of physiological importance not only on account of the amino acids, but also be-

[6] Niels Bjerrum (1879–) is Professor of Chemistry in the Royal Veterinary and Agricultural College, Copenhagen, Denmark. He has done important research work on the electrochemistry of solutions.

cause the proteins are amphoteric electrolytes. In the presence of bases, proteins react like weak acids; in the presence of acids, they react like weak bases; each protein has its own isoelectric point, and may most readily be freed from combination with ions if it is brought to its isoelectric point by the addition of the necessary small amount of acid or base (76). The applications of physico-chemical theory to proteins and amino acids are presented in the review articles by Cohn (29, 30).

Brønsted's Conception of Acids and Bases. The ionization equilibria of acids and bases are formulated by Brønsted[7] (20) in a modified form which has the advantages of simplicity and of general applicability to solutions in any solvent. An acid is defined as any molecule or ion which can give off a hydrogen ion in solution, and a base is an ion or molecule which can combine with a hydrogen ion to form an acid.

$$\underset{\text{acid}}{A} \rightleftharpoons H^+ + \underset{\text{base}}{B^-}.$$

Each acid is related to a conjugate base, and *vice versa*. A base is always more negative by one electron than its conjugate acid. An acid need not be electrically neutral, and a base is not always a negative ion. Thus NH_4^+ and CH_3COOH are acids whose conjugate bases are NH_3 and CH_3COO^-. Water is both an acid and a base; it may lose H^+ to form the base OH^-, or it may combine with H^+ to form the hydrated hydrogen ion, H_3O^+, which is an acid. Any acidic dissociation constant is the reciprocal of the association constant of the conjugate base.

With this system of notation, both dissociation constants of a simple ampholyte may be treated as constants of acidic dissociation, the cation of the ampholyte being considered as a dibasic acid. This treatment is identical with that of Adams (4), and is in accord with the *Zwitterion* theory of Bjerrum (17), who has adopted Brønsted's notation in later work.

[7] Johannes Nicolaus Brønsted (1879–) is Professor of Chemistry in the University of Copenhagen, Denmark. He is known for his research work on chemical affinity, theories of electrolytes, and catalysis.

PROBLEMS

1. Derive the relation $\frac{\Lambda^2 C}{\Lambda_0(\Lambda_0-\Lambda)}=K_a$ for a weak acid.

2. Use the following data to test the applicability of the Ostwald dilution law to acetic acid at 18°:

C	0	0.01	0.1	1.0
Λ	348	14.5	4.68	1.34

3. From the data given in the preceding problem, calculate the degree of ionization and equivalent conductivity of 0.001 *M* acetic acid.

4. Calculate (H^+) in 0.1 *M* acetic acid, and in a solution which is 0.1 *M* with respect both to acetic acid and sodium acetate.

5. If $K_s=2.4\times10^{-10}$ for AgCl, calculate its solubility in pure water and in 0.01 *M* HCl.

6. Calculate (H^+) in 0.1 *M* NH_4Cl if $K_b=1.8\times10^{-5}$ for NH_4OH.

7. Calculate the isoelectric point of an ampholyte for which $K_a=7.8\times10^{-10}$ and $K_b=1.6\times10^{-12}$.

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52. HAMMETT, Chapters I–VI.
97. NOYES[8] and SHERRILL, Chapter III.
127. WASHBURN, Chapters XV, XVII, XXII, XXIII.

[8] Arthur Amos Noyes (1866–), of the California Institute of Technology, formerly Professor of Theoretical Chemistry at the Massachusetts Institute of Technology, is known for his applications of physical chemistry to analytical separations, and for his contributions to the theory of solutions of electrolytes.

CHAPTER V

Hydrogen Ions, Indicators, and Buffers

The Reaction or Acidity of Solutions. Since water is the universal solvent in living matter, and most biochemical reactions take place in aqueous solution, the ions of water have peculiar significance in physiology. In studying this significance it is customary to express the results in terms of the concentration (more correctly, the activity) of hydrogen ions. Since, for aqueous solutions, (H^+) and (OH^-) are related by the law of the constancy of the ion product K_w, the reaction (acidity or alkalinity) of a solution could just as well be expressed in terms of (OH^-), or both terms could be used in different cases. Ordinarily, for the sake of uniformity, (H^+) is used in referring to the reaction of any solution. A 0.1 *M* NaOH solution is strongly alkaline, the concentration of hydroxyl ions being about 0.1 *M*; yet this fact may be described just as definitely by stating that for this solution $(H^+) = 1 \times 10^{-13}$. The reaction of any solution may be precisely expressed in terms of the concentration of hydrogen ions, no matter whether that quantity be a large or a small number.

The value of (H^+) in an acid solution is usually very different from the normality of the total acid, as found by titration. For a dilute solution containing only a single strong acid the two quantities are practically identical, but for any solution containing a weak acid the normality as found by titration is much greater than the actual acidity, which is the activity or concentration of hydrogen ions. Similar considerations apply to the total normality and hydroxyl ion activity or concentration of alkaline solutions.

Hydrogen Ion Exponent or pH. Another mode of expressing acidity has become widely used, particularly in biochemical work. In the experimental determination of hydrogen ion activity by the electromotive force method (which is to be discussed later), the quantity measured is proportional, not to

(H^+) itself, but to its logarithm. The numerical values of (H^+) in ordinary solutions cover an extremely wide range; for a normal solution of a strong acid (H^+) is nearly 1, while for a normal solution of a strong base (H^+) is about 1×10^{-14}. Such a wide range of numbers may be expressed in less space, either when written or plotted graphically, if not only the power of 10 but also the number by which it is multiplied is expressed as an exponent or logarithm. It is also true that the values of (H^+) in most ordinary solutions are decimals or small fractions. The logarithm of a fraction is a negative number. For these reasons Sørensen[1] in 1909 proposed that hydrogen ion concentration be expressed in terms of its negative logarithm, which is the same as the logarithm of its reciprocal; and for this quantity the symbol pH has been generally adopted. It may be defined by the equation

$$\text{pH} = -\log (H^+) = \log \frac{1}{(H^+)}$$

where (H^+) refers to the activity of hydrogen ions in the solution. The relation between (H^+) and pH is simple enough when the values of (H^+) are integral powers of 10, as the following table shows:

(H^+)		pH
10 ..... or	10^{+1}	−1
1.	10^{0}	0
0.1	10^{-1}	1
0.01	10^{-2}	2
0.0000001	10^{-7}	7
0.0000000001	10^{-10}	10

For intermediate values it is necessary to find the logarithm of (H^+), and then write its negative value. For example, if

[1] Søren Peter Lauritz Sørensen (1868–) is one of the best known of living biochemists. He is Director of the Chemical Department of the Carlsberg Laboratory in Copenhagen, in which post he succeeded Kjeldahl, of nitrogen fame. Sørensen was one of the first to appreciate the significance of hydrogen ions in biology, and he has done beautiful quantitative work in applying physico-chemical methods to the study of enzymes and proteins.

$(H^+) = 2 \times 10^{-5}$, $\log (H^+) = \log 2 + \log (10^{-5}) = 0.3 - 5.0 = -4.7$, and $pH = 4.7$, without the minus sign. Similarly if $(H^+) = 4 \times 10^{-10}$, $\log (H^+) = \log 4 + \log (10^{-10}) = 0.6 - 10.0 = -9.4$, and $pH = 9.4$. While these calculations may seem awkward to one who has forgotten the meaning of a logarithm, they can be made easily after a little practice with a short table or a slide rule. Considerable time may be saved in calculation if it is remembered that the accuracy of experimental pH determinations is so limited that one is not justified in keeping more than two figures in the mantissa of the logarithm, except in the case of extremely refined measurements, when three figures may be justified.

Qualitatively a high value of pH means a low value of (H^+), or an alkaline solution. A low pH value means high acidity. The pH of the neutral point is 7.0, since $(H^+) = 1 \times 10^{-7}$ in pure water. A 0.1 *M* solution of a strong acid has a pH value of about 1, and for a 0.1 *M* solution of a strong base, pH is about 13.

Titration Curves. If it be granted, for the moment, that it is possible to measure pH, it may be instructive to examine the changes in pH occurring in some of the simple titrations of analytical chemistry. Fig. 7 shows that in the titration of a strong acid with a strong base, there is a tremendous change in pH at the point of equivalence. The curve is vertical for a distance corresponding to nearly 4 pH units, or a change in (H^+) of ten thousandfold. This is in agreement with the fact that in such a titration in volumetric analysis the same end point is obtained with almost any of the ordinary indicators. In titrating a weaker acid such as acetic, however, the jump in pH at the end point is much smaller. This explains the fact that for good results by the ordinary volumetric method an indicator must be chosen which exhibits its color change in the pH range corresponding to the vertical part of the curve. For this particular titration, phenolphthalein is such an indicator. Similar considerations hold for the titration of the weak base ammonium hydroxide with the strong acid, hydrochloric acid. Here the vertical part of the curve falls between pH 4.5 and 7.0, which corresponds with the empirical fact that methyl red, which

changes color in this region, is a good indicator for this particular titration. The curve for the titration of acetic acid with ammonium hydroxide shows that there is no sharp break at the end point when both acid and base are weak.[2]

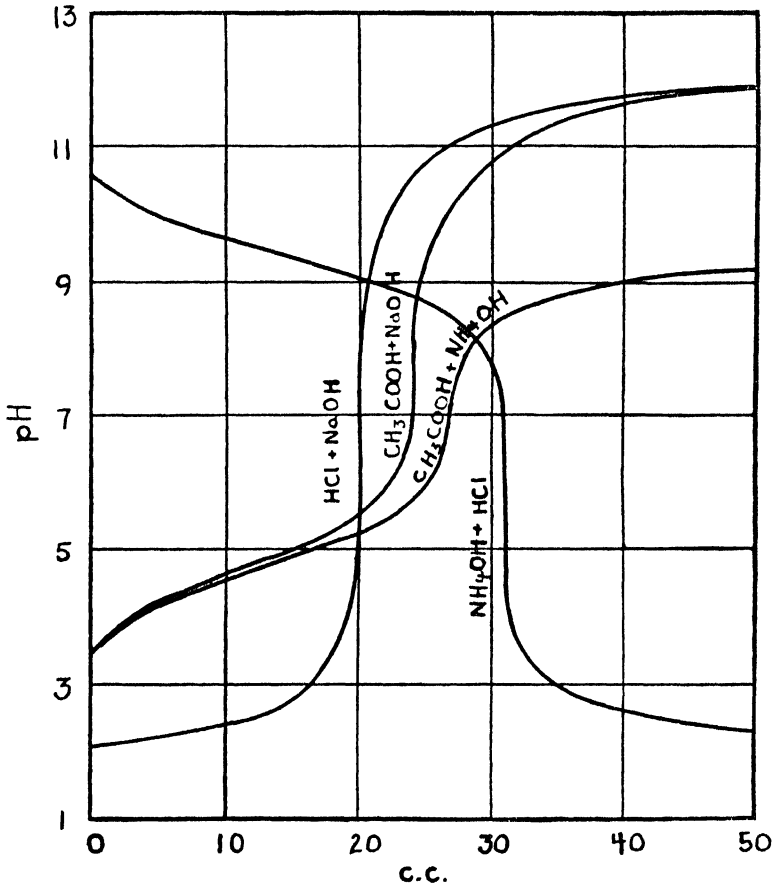

FIG. 7. Titration curves of weak and strong acids and bases.

Indicators. An indicator is a weak acid, base or ampholyte whose ionized and un-ionized forms have different colors. The

[2] The curves were obtained by titrating 25 cc. of approximately 0.1 *M* acid or base, diluted with 200 cc. of water, with approximately 0.1 *M* base or acid. The concentrations of the solutions differed enough from 0.1 *M* so that the end points do not fall at 25 cc. in each case.

usefulness of an indicator depends on the ease with which these colors may be distinguished and on the particular range of pH values in which its color changes take place. To be useful in the ordinary titrations of analytical chemistry, the indicator should have a sharp color change in the region of pH where the titration curve of the reacting substances is parallel to the pH axis, as illustrated in Fig. 7. For use in the colorimetric determination of pH values, comparison is made of the finer gradations in the color of the indicator.

Most indicators behave like weak mono-basic acids. Neglecting possible tautomeric forms, the ionization of such an indicator may be written

$$HIn \rightleftharpoons H^+ + In^-$$

and the law of mass action may be applied to this reaction, giving

$$(H^+) = \frac{(HIn)}{(In^-)} K_a$$

or

$$pH = pK_a + \log \frac{(In^-)}{(HIn)} \cdot \qquad (1)$$

The symbol pK_a means $-\log K_a$, just as pH is $-\log (H^+)$. The ratio of the concentrations of the ionized and un-ionized forms is the ratio of that fraction of the indicator in the ionized or alkaline form to that in the un-ionized or acid form. If the fraction in the alkaline form is denoted by α, equation (1) becomes

$$pH = pK_a + \log \frac{\alpha}{1 - \alpha} \cdot \qquad (2)$$

Fig. 8 shows a graph of equation (2), α being plotted against the difference between pH and pK. If the indicator has only one color, say in the alkaline form, then α, or the fraction of the indicator in the colored form, may be determined for any solution by a colorimetric comparison with a solution containing the same concentration of indicator which is all known to be in the colored form. Equation (2) has in this way been verified for certain indicators.

Colorimetric Determination of pH without Buffers. A simple way of testing equation (2) is applicable also to indicators which are colored in both forms. Any intermediate color of an indicator may be considered to be produced by the presence of a certain number of particles which have the full acid color, and a certain number of other particles which have the full alkaline color. If a solution of known pH produces a certain intermediate

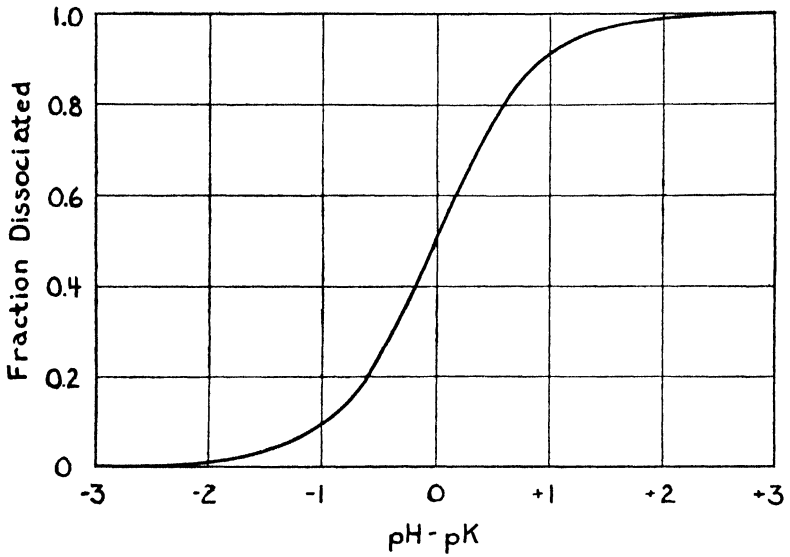

FIG. 8. Dissociation curve of a weak acid, a buffer mixture, or an acidic indicator.

color of the indicator, when, for example, 10 drops of indicator are added to 10 ml. of solution, it is possible to match that color by distributing 10 drops of the same indicator between 10 ml. samples of two solutions, of which one is acid enough to transform all of the indicator into the acid form, and the other alkaline enough to effect the reverse transformation. The colors are compared by having the solutions in test tubes in a comparator block of the form shown in Fig. 9. On looking through the holes in the block, light reaches the eye through the standard tube containing 10 drops of indicator, backed by a tube of water, and through the tubes of acid and alkali in which 10 drops of indi-

cator are divided between the two tubes. When, by varying the numbers of drops of indicator in the acid and alkali tubes, a color is produced which matches the standard, then it may be said that the indicator in the standard solution of known pH has the same ratio of alkaline form to acid form as the numbers of drops of indicator in the alkaline and acid tubes. For example, if a match is obtained with 3 drops in the alkaline tube and 7 in the acid, the value of $\alpha/(1-\alpha)$ for the indicator in the standard solution may be said to be $3/7$, or 0.43. By using this value in equation (2), it is possible to calculate the apparent value of

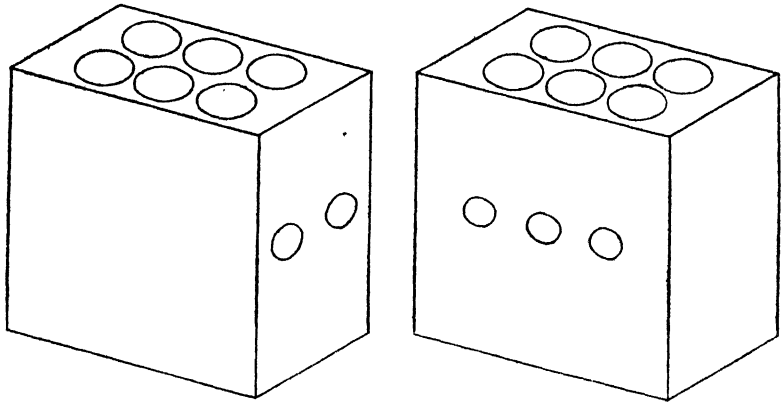

FIG. 9. Comparator blocks.

pK_a for the indicator under the conditions of the experiment if the pH of the standard solution is known. By using several standard solutions of varied pH, it is possible to test equation (2) for any indicator. If the equation applies, the value of pK_a will be constant and independent of pH. This has been done for a number of indicators, and the equation has been found, in general, to hold very well. One exception is phenolphthalein, which behaves as a dibasic acid. The following table gives the pK_a values obtained in this way by Clark[3] (26) and others for a number of indicators.

[3] William Mansfield Clark (1884–), Professor of Physiological Chemistry in the Johns Hopkins University, has done much to increase the precision of biochemical work by his studies of indicators, buffers, and oxidation-reduction potentials. He was formerly Professor of Chemistry at the Hygienic Laboratory of the United States Public Health Service.

Indicator	pK_a
Thymol blue (acid range)	1.5
Brom phenol blue	4.0
Brom cresol green	4.7
Methyl red	5.1
Chlor phenol red	6.0
Brom cresol purple	6.2
Brom thymol blue	7.0
Phenol red	7.8
Cresol red	8.3
Thymol blue	8.9

By the use of these values, which hold for about 20° C., it is possible to make determinations of pH by the use of indicators without any standard solutions of known pH (47). The method is not particularly exact, as may be inferred from the fact that different workers have reported pK_a values for the same indicator differing by as much as 0.2 units. It has been refined by the use of more dilute indicator solutions which can be measured by a calibrated micro burette instead of by drops, and under carefully controlled conditions it has been shown to be accurate to 0.02 or 0.03 pH for determination of the pH of blood serum and urine (57). Even in its simplest form it is a very instructive method because it furnishes a simple means of testing the theoretical equation (2).

Colorimetric Determination of pH with Buffers. If a set of standard solutions of known pH is available, the pH of an unknown solution may be determined by taking equal volumes of the standard and unknown solutions, adding the same amount of the same indicator to each, and comparing the resultant colors. If the initial solution is clear and colorless, no comparator block is needed. Solutions of the same pH will impart the same color to the indicator. A large number of standard solutions may be required, and it is of course necessary to use an indicator which is, at the pH of the unknown solution, not wholly transformed either into its acid or alkaline form. The useful range of any one indicator is not more than 2 pH units.

In a solution which is colored or turbid, the appearance of an indicator is not the same as in a clear colorless solution. In such

cases the pH may be estimated by using the comparator block (Fig. 9), looking at the unknown through a tube of water and at the standard through a tube of the colored or turbid unknown without indicator. If such solutions are to be studied by the indicator method without buffers, a comparator block with three rows of holes is required.

The colorimetric method is not one of the highest accuracy. With standards 0.1 pH apart, it should be possible to estimate differences of about 0.03 pH, but the uncertainty is much greater with colored or turbid solutions. Even this accuracy cannot be claimed for unknown solutions in general, because the colors of indicators are perceptibly influenced by salts and proteins in solution. The ultimate standard in all pH determinations is the hydrogen electrode method.

Buffer Solutions. In the preceding descriptions of colorimetric methods for pH determination, the existence of stable standard solutions of known pH has been implied. Such solutions owe their stability of pH to what is known as buffer action. A buffer is a solution which tends to resist changes in pH; it has reserve acidity and alkalinity, or it can take up appreciable amounts of acid or alkali with only slight changes in pH. A concentrated solution of a strong acid or base is a good buffer, while a dilute solution of the same is a very poor one, as is distilled water. Usually a buffer is defined as a solution containing a weak acid or base with one of its salts. Approximate values of (H^+) or pH for such solutions may be calculated from the mass law if the composition of the mixture and the ionization constant of the weak electrolyte are known. For example, in the case of a weak acid such as acetic,

$$(H^+) = K_a \frac{(HA)}{(A^-)} \cdot \qquad (3)$$

In a mixture of HA + NaA, most of the acid is un-ionized, so that (HA) is approximately equal to the total concentration of acid. Since the acid is slightly ionized and the salt completely ionized, the value of (A^-) is approximately equal to the total salt concentration. Accordingly, for approximate calculations,

the equation may be written

$$(H^+) = K_a \frac{(\text{acid})}{(\text{salt})} \cdot \tag{4}$$

This approximate form of the mass law is sometimes mentioned in biochemical literature as Henderson's equation. When expressed in terms of pH it becomes

$$\text{pH} = \text{p}K_a + \log \frac{(\text{salt})}{(\text{acid})} \tag{5}$$

which is sometimes called the Henderson-Hasselbalch equation. These equations are useful for approximate calculations of pH in buffer mixtures.

Dissociation Curves. Another form of the mass law is obtained by defining a degree of dissociation, α, for a buffer mixture as that fraction of the total acid radical, ionized+unionized, which is present in ionic form, or

$$\alpha = \frac{(A^-)}{(HA) + (A^-)} \cdot \tag{6}$$

It will be noted that this expression has the same form as one of the equations for the degree of dissociation of a weak acid in pure water (Chapter IV, eq. (7)). In a buffer mixture, however, (A^-) and (H^+) are not equal, since most of the A^- ion comes from the added salt, while (H^+) is low because of the slight dissociation of the weak acid. A combination of equations (3) and (6) gives

$$\alpha = \frac{K_a}{K_a + (H^+)} \tag{7}$$

or

$$(H^+) = K_a \frac{1 - \alpha}{\alpha} \tag{8}$$

or

$$\text{pH} = \text{p}K_a + \log \frac{\alpha}{1 - \alpha} \tag{2}$$

which is the same equation already derived for the special case of an indicator that behaves as a weak acid. A graphical representation of these equations is given in Fig. 8, in which α is plotted against change in pH. Curves of this type are called dissociation curves. From equation (7) it may be seen that when (H^+) is very small, α approaches 1, and when (H^+) is very large, α approaches zero. When $\alpha = 0.5$, equation (8) shows that $(H^+) = K_a$. These properties of the relation between α and (H^+) are all depicted in Fig. 8. It is to be noted that all weak acids have dissociation curves of exactly this shape, the only difference in the case of weak acids of different strengths being in the location along the pH axis. The mid-point of each curve is always so located that its pH is equal to pK_a for that particular acid. Since the definition of α for a buffer mixture applies also to the degree of dissociation of a weak acid in the absence of salt, equations (2, 7, 8) and Fig. 8 apply also to solutions of a weak acid in water, in so far as it may be possible to vary the pH by simple dilution rather than by partial neutralization.

By applying the mass law to the dissociation of a weak base, relations similar to equations (2, 7, 8) may be obtained. If α for a weak base is plotted against pH, the curve has the same shape as that in Fig. 8, but slopes in the opposite direction. The curve of the fraction undissociated $(1 - \alpha)$ for a weak base has exactly the same shape and direction as the dissociation curve of a weak acid. The curves of α and $1 - \alpha$ for any weak electrolyte intersect at their mid-points. In the case of a weak base the pH value at the mid-point of these curves is not the value of pK_b, but of $pK_w - pK_b$.

An experimental titration curve for a weak acid, such as the curve for acetic acid in Fig. 7, is made up in part of a curve which is nearly identical with a theoretical dissociation curve. The resemblance becomes closer if the titration is carried out at constant volume; that is, by measuring the pH of several solutions containing the same amount of acid but varied amounts of alkali, and all diluted to the same volume. As long as there is an excess of acid, all of the alkali added may be considered to be neutralized, setting free the anion from an amount of the acid

equivalent to the alkali, so that the volume of alkali added is practically proportional to α, the ionized or neutralized fraction of the total acid. By means of experimental curves constructed in this way it has been possible to show that the theoretical curves and equations fit the facts quite well. Even better agreement is obtained if the solutions are so made up as to have a constant salt content, as measured by the ionic strength (75, 49).

The action of buffers in resisting changes in pH may be explained by a consideration of the curve in Fig. 8 or its equation. The addition of acid or base to a buffer mixture must change the value of α. The buffer will be most effective where its pH changes least for a given change in α. The figure shows that this is the case at the mid-point of the curve; here it has a point of inflection and its slope is steepest. The buffer value is poor at either end of the curve, where it becomes asymptotic with the pH axis. Practically, the curve is straight enough so that a buffer is fairly efficient at any pH value not more than 1 unit removed from the value of pK_a of the weak acid. For most efficient buffering at any pH, the weak acid of the buffer should be so selected that its pK_a is equal to the pH to be maintained, and it should be used in a solution with an equivalent amount of one of its salts, or the acid should be just half neutralized by a strong base.

The fact that all weak acids have dissociation curves of the same shape implies that all buffers are equally efficient, if compared at a fixed value of α. This is not at all true, however, if they are compared at a fixed pH. At a pH value more than about 3 units distant from its pK_a, a buffer mixture is not appreciably more effective in resisting changes in pH than is a very dilute solution of a strong acid or base having the same pH. This is shown in the titration curves in Fig. 7; if the sodium acetate or the ammonium chloride were still an effective buffer at the point of equivalence, the latter would not be marked by a sudden change in pH.

Obviously a concentrated buffer solution will resist changes in pH more effectively than a dilute one, not because it has a different dissociation curve, but because a given amount of

added acid or base will have less effect on α, the dissociated fraction of the buffer acid.

Biological Applications. Measurements of hydrogen ion concentration have been most fruitful in biochemistry in studies of enzyme action, the behavior of proteins, and the equilibrium between acids and bases in the blood. All of these subjects are discussed in later chapters of this book.

PROBLEMS

1. If the method of pH measurement without buffers is used with 10 drops of indicator distributed in ratios of 9:1, 8:2, and so on down to 1:9, calculate the differences in pH corresponding to the differences in color between the various pairs of tubes.

2. It is desired to prepare a buffer mixture having a pH of 5.3. The only weak acids available are two, having dissociation constants equal to 10^{-4} and 10^{-7}. Which one would be selected, and what ratio of the acid to its salt would be used?

3. A 0.2 *M* solution of acetic acid was half neutralized by NaOH. The pH of this mixture was found to be 4.62. Calculate the pH of a 0.2 *M* solution of acetic acid which has been only one-quarter neutralized by NaOH.

4. If the equilibrium constant for the reaction

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

is 7.6×10^{-7}, and the pH of blood serum is 7.4, calculate the ratio of bicarbonate to carbonic acid in the serum.

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CHAPTER VI

Galvanic Cells and Electrometric pH Determination

It has been stated in the previous discussion of hydrogen ion concentration or activity that the measurement of this quantity depends ultimately on the use of the hydrogen electrode. In order to explain how this and other electrodes behave, and to give an idea of the significance of such electrode measurements, a brief excursion into the field of chemical thermodynamics is necessary.

Free Energy and Equilibrium Constant. If a chemical reaction proceeds spontaneously under a given set of conditions, that reaction is capable of doing work. The maximum work available from a reaction which takes place at constant temperature and pressure is called the free energy change of the reaction, and it is related to the equilibrium constant of the reaction and, for a reaction between highly diluted substances, to the initial concentrations of the reacting substances. For a spontaneous reaction the free energy change is a decrease, and is represented by the symbol $-\Delta F$. For any reaction in general of the type

$$m\mathrm{A} + n\mathrm{B} \rightleftharpoons p\mathrm{C} + q\mathrm{D},$$

the relation in question has the form

$$-\Delta F = RT \ln K - RT \ln \frac{(\mathrm{C})^p(\mathrm{D})^q}{(\mathrm{A})^m(\mathrm{B})^n}. \qquad (1)$$

It will be recalled that the equilibrium constant K is equal to a ratio of exactly the same form as that in the last term of equation (1). The value of K is a ratio of equilibrium concentrations, while the ratio in the last term is a ratio of initial concentrations. The free energy decrease for a mixture of substances at equilibrium is zero; such a mixture is not capable of doing any work. For a mixture not in equilibrium, the free energy decrease measures the work which the system could do, under ideal or thermodynamically reversible conditions, in getting to the equilibrium condition.

Experimentally it has been found that equation (1), like the law of mass action, does not hold exactly for reactions between gases at rather high pressures, or between solutes in any but very dilute solutions, if the parentheses are taken to refer to partial pressures or volume concentrations or molalities. For this reason Lewis[1] (1907) defined a new function, the activity, in such a way that if it is substituted for concentration in the law of mass action and in equation (1) the equations are exact for all concentrations and the values of K are truly constant.

Free Energy and Electromotive Force. If a chemical reaction can be made to take place in a galvanic cell, it may be possible to arrange matters so that the change in free energy appears as electrical work. In order for this to be true it is necessary to have a cell with reversible and non-polarizable electrodes. Such an electrode may consist of almost any metallic element dipping into a solution containing one of its salts. Other examples are electrodes of an inert metal such as platinum, coated with platinum black, saturated with a gas such as hydrogen or chlorine, and immersed in a solution containing hydrogen ion or chloride ion. Such electrodes are known as electrodes of the first class; it is characteristic of this class that the element forming the electrode is in contact with a solution containing the same element in ionic form. The electrode reaction is the transfer of electrons from the substance of the electrode to its ions in the solution, or the reverse. Such a reaction may be conceived to have a spontaneous tendency to take place, to a very slight extent, at a single electrode, but the extent of the reaction is limited by the electrostatic forces set up by this electron transfer. A galvanic cell must have two electrodes, and the cell reaction is the algebraic sum of the two electrode reactions. If the cell is allowed to deliver current, or if a current is passed

[1] Gilbert Newton Lewis (1875–), of the University of California, is one of the leading physical chemists of the present day. Early in his career he held a government scientific post in the Philippines, and was instrumental in establishing the Metric System in that country. He is particularly well known for his studies of free energy in chemical reactions. The book by Lewis and Randall (75) is the best modern work on chemical thermodynamics.

through it from some outside source, the cell reaction goes on, in one direction or the other, to an extent limited only by the duration of the flow of current.

If the electromotive force of a galvanic cell is exactly balanced by an opposing electromotive force from an outside source, no current will flow. An infinitesimal change in the external electromotive force causes the cell reaction to proceed, in one direction or the other, and causes an infinitesimal current to flow through the cell. If the external electromotive force is brought back exactly to its original value, the cell reaction must be reversed and current must flow in the opposite direction until the cell is exactly in the same condition as at the start. If a cell responds in this way to infinitesimal changes imposed upon it from outside, the cell and its electrodes are said to be reversible. Reversibility in this thermodynamic sense is an ideal condition never actually attained in practice, but it is closely approached in the measurement of the electromotive force of a suitable galvanic cell by the potentiometer method. (This method is described later in this chapter.)

If current is drawn from a cell, or passed through it, for more than an infinitesimal time, the cell becomes polarized. Polarization is the production by the passage of current of any change in the solution touching the electrode or in the surface of the electrode which alters the electromotive force of the cell. Polarization may occur as a result of changes in the concentration of the solution or as a result of the formation of a film of gas on the surface of the electrode. Any cell will become polarized if a large current is drawn from it. During the passage of a small current, polarization due to concentration changes may be minimized by keeping the solution saturated with the electrolyte, and polarization due to the formation of gas may be minimized by having the surface of the electrode coated with finely divided metal such as platinum black, which absorbs large quantities of gases and acts as a catalyst for the interchange of electrons between a gas and its ions in solution. The hydrogen electrode is considered to be non-polarizable in the latter sense if kept saturated with hydrogen gas. An electrode of zinc in a

saturated solution of zinc sulphate is non-polarizable in the sense that small currents will not change its potential by means of concentration changes, because the presence of the solid salt keeps the concentration constant and equal to that of a saturated solution.

A reversible electrode of the second class usually consists of a metal in contact with a slightly soluble salt of the same metal, dipping into a solution containing the anion of the slightly soluble salt. Such an electrode is reversible with respect to the anion, even though the elementary or uncharged form of the anion is not present. The anion may consist of several atoms, so that its uncharged form may not exist. For example, an electrode of metallic mercury in contact with solid mercurous sulphate in a solution of a soluble sulphate is reversible with respect to the sulphate ion. Other commonly used electrodes of this class are the calomel electrode, consisting of mercury in contact with solid mercurous chloride (calomel) in a solution of a soluble chloride, and the silver-silver chloride electrode, consisting of silver coated with silver chloride and dipping into a solution of a soluble chloride. These two electrodes are reversible to the chloride ion. In the last analysis the electrode reaction is still the interchange of electrons between the metal and its ion, since that part of the solution near the metal is saturated with the slightly soluble salt. The activity of this salt in the solution is constant at any constant temperature, and therefore the activity product of its ions is a constant. The activity of the metallic ion varies inversely as the activity of the anion, no matter what soluble electrolyte may be used to furnish this anion.

For a cell composed of two reversible electrodes in a single solution, if the electromotive force is measured without drawing appreciable current from the cell, the free energy decrease of the cell reaction is related to the electromotive force by the equation

$$-\Delta F = NE\mathbf{F}. \qquad (2)$$

Here E is the electromotive force of the cell in volts, $\mathbf{F}$ is the

faraday,[2] or the number of coulombs (96,500) of electricity associated with one gram-equivalent of ions in solution, and N is the number of chemical equivalents involved in the reaction at each electrode when the chemical reaction proceeds as written. The free energy change $-\Delta F$ is obtained in electrical units; namely, volt-coulombs or joules.

Electromotive Force and Activity of Electrolytes. Consider a cell composed of an electrode of silver, coated with silver chloride, and a hydrogen electrode, both dipping into the same solution of hydrochloric acid. Such a cell is usually written, following the convention of Lewis, with the negative electrode at the left, or

$$H_2,\ HCl,\ AgCl,\ Ag(+).$$

For the cell so written, the electromotive force is given a positive sign. The cell reaction corresponding to the passage of one faraday of electrons from right to left inside the cell is

$$\underset{\text{gas}}{\tfrac{1}{2}H_2} + \underset{\text{solid}}{AgCl} \rightleftharpoons \underset{\text{solid}}{Ag} + H^+ + Cl^-.$$

If equations (1) and (2) are applied to this cell reaction, for which $N = 1$, the E.M.F. should be

$$E = \frac{RT}{N\mathbf{F}} \ln K - \frac{RT}{N\mathbf{F}} \ln \frac{(Ag)(H^+)(Cl^-)}{(H_2)^{1/2}(AgCl)}$$

where the parentheses are understood to represent activities. The activity of a solid is a constant, just as its concentration is, since it has a definite density or mass per unit volume. If the hydrogen gas is at a constant pressure of 1 atmosphere, its activity also is constant. By collecting these constants with the constant term $(RT/N\mathbf{F})\ln K$, the equation may be re-written

[2] This unit is named after Michael Faraday (1791-1867), who was the son of an English blacksmith. He became assistant to Humphry Davy at the Royal Institution, London, and was later Professor of Chemistry there. He isolated benzene, and discovered electromagnetic induction, the laws of electrolysis, and the effect of a magnetic field on polarized light. He also wrote on such diverse subjects as the liquefaction of gases, on holding the breath for a lengthened period, and the passivity of iron in nitric acid. He was one of the greatest of scientific discoverers.

$$E = E_0 - \frac{RT}{N\mathbf{F}} \ln (H^+)(Cl^-). \qquad (3)$$

Evidently the constant term E_0 represents the value which E would have if the product of the activities of H^+ and Cl^- were one. In this equation R must be expressed in electrical units, which are volt-coulombs or joules per degree, and in these units $R = 8.316$. Changing to logarithms to the base 10, equation (3) becomes

$$E = E_0 - 0.0001984T \log (H^+)(Cl^-), \qquad (4)$$

or, for 25° C.,

$$\frac{E - E_0}{0.05915} = - \log (H^+)(Cl^-). \qquad (5)$$

By measuring the E.M.F. of such cells with varied concentrations of hydrochloric acid, it is possible to obtain a value for E_0 by extrapolation, making use of the fact that at infinite dilution, as the concentration approaches zero, the activity becomes equal to the molality. Equation (5) therefore furnishes a means of obtaining from electromotive force measurements values for the product of the activities of the ions of a strong electrolyte. Strictly it is only this activity product which can be determined with certainty from any such electromotive force measurements. Since there is no reason to believe that the activities of the individual ions in a solution such as hydrochloric acid are exactly equal, it is not possible to measure, without further assumptions, the activity of a single ion such as the hydrogen ion.

Lewis defined the mean activity of the ions of a uni-univalent electrolyte as the square root of the product of the activities of its ions. The activity coefficient is defined as the factor by which the geometric mean molality of the ions must be multiplied to give the mean activity, or, for a uni-univalent electrolyte,

$$\gamma = \frac{\sqrt{a_+ a_-}}{m}$$

where a refers to the activity of an ion, m is the molality of the electrolyte, and γ is the geometric mean activity coefficient of

the ions. It is sometimes convenient to talk about the activity coefficient of a single ion such as H^+, which is

$$\gamma_H = \frac{a_H}{m_H}$$

where m_H is the total molality in the case of a strong acid such as hydrochloric, and the molality of H^+ ions in the case of a weak acid such as acetic. In the latter case γ_H is probably very close to one, since the mass law holds fairly well for ion concentrations as calculated from conductivity.

Concentration Cells. If two cells such as those described in the preceding section are connected in series with like poles together, the E.M.F. will be zero if the concentration of hydrochloric acid in both cells is the same. If the concentrations are not the same, there will be a definite E.M.F. The net cell reaction which takes place when one faraday of electrons is delivered by the combined cell is the transfer of one equivalent of H^+ and of Cl^- from the more concentrated to the more dilute solution. Such a double cell is called a concentration cell without liquid junction (or without transference), and its E.M.F. is a measure of the decrease in free energy accompanying the dilution of the electrolyte. The E.M.F. of such a cell is given by the relation

$$E = \frac{RT}{N\mathbf{F}} \ln \frac{a_H' a_{Cl}'}{a_H'' a_{Cl}''} = \frac{2RT}{N\mathbf{F}} \ln \frac{m'\gamma'}{m''\gamma''} \cdot \qquad (6)$$

If the E.M.F. is considered positive, the single primes refer to the more concentrated solution and the double primes to the more dilute. The symbol γ refers to the geometric mean activity coefficient of the two ions.

Another type of concentration cell is composed of two identical electrodes dipping into two solutions of the same electrolyte of different concentrations. Such a cell is called a concentration cell with liquid junction, or with transference. Examples of such cells are

$$\text{Ag, AgCl, HCl } (m'), \text{ HCl } (m''), \text{ AgCl, Ag } (+ \text{ if } m' > m'')$$

and

$$H_2,\ HCl\ (m''),\ HCl\ (m'),\ H_2\ (+ \text{ if } m' > m'').$$

The E.M.F. of these cells is given by the equation

$$E = \frac{tRT}{\mathbf{F}} \ln \frac{a_H' a_{Cl}'}{a_H'' a_{Cl}''} = \frac{2tRT}{\mathbf{F}} \ln \frac{m'\gamma'}{m''\gamma''} \qquad (7)$$

where t is the transference number[3] of the ion to which the electrodes are not reversible and the other symbols have the same meaning as in equation (6). Equation (7) holds only for solutions for which t may be considered constant for different concentrations. A more general equation is given in larger works ((75), pp. 337–340; (55), pp. 813–5).

Concentration cells of these types, involving no liquid junction or a junction between two solutions of the same electrolyte, have extremely constant and reproducible electromotive force values. Such cells have so far been used largely with inorganic electrolytes, but Harned[4] (56) and others (66) have applied them to certain solutions of more or less biological interest.

Liquid Junction Potentials. A potential difference exists across the junction of any two electrolytic solutions which are in contact, due to the unequal mobilities or diffusion velocities of the different ion species. For solutions of the same electrolyte at different concentrations, such potential differences are relatively small, and can be approximately calculated. Such a liquid junction potential or diffusion potential is included in the E.M.F. given by equation (7) for a concentration cell with transference. There is, however, no exact way to calculate the junction po-

[3] This is the fraction of the current carried by one ion species of an electrolyte during electrolysis. For an explanation of its real significance and its relation to ionic mobilities, reference must be made to larger texts of physical chemistry.

[4] Herbert Spencer Harned (1888-), formerly of the University of Pennsylvania and now Professor of Chemistry in Yale University, is one of the most active of contemporary physical chemists. His work has dealt largely with the electrochemistry of solutions. His summary of this subject (55) is heartily recommended to ambitious students.

tential between any two solutions in general, and the magnitude of such potentials between solutions of different electrolytes seems to vary with the method of making the junction. The best measurements of the E.M.F. of cells involving such junction potentials have been made by the use of flowing junctions (71, 104).

It happens that the ions of potassium chloride have nearly equal atomic weights and mobilities. Accordingly if a concentrated solution of potassium chloride is in contact with a dilute solution of an electrolyte, it is to be expected that most of the current passing across the boundary will be carried by the K^+ and Cl^- ions, owing to their high concentration, and that diffusion potentials will be small owing to the nearly equal mobilities of these ions. This conclusion has never been proved, and for that reason most physical chemists have given up the use of "salt bridges" as a means of eliminating diffusion potentials. Yet measurements involving such junctions form the basis of all measurements of pH, and the results have been of sufficient significance so that the method is still in wide use, particularly among biochemists.

Determination of pH by the Hydrogen Electrode. The basis of all electrometric pH measurement is the following cell:

H_2, standard solution, KCl (sat.), unknown solution, H_2.

The E.M.F. of such a cell cannot be calculated by thermodynamics. It is assumed, however, that the liquid junction potentials between the potassium chloride and the standard and unknown solutions are equal and opposite in direction, so that they cancel one another. If this assumption is granted, it can be shown that the E.M.F. of the cell should be given by the equation

$$E = \frac{RT}{N\mathbf{F}} \ln \frac{a_{\mathrm{H}}'}{a_{\mathrm{H}}''} = 0.0001984 \frac{T}{N} \log \frac{m_{\mathrm{H}}'\gamma_{\mathrm{H}}'}{m_{\mathrm{H}}''\gamma_{\mathrm{H}}''} \cdot \qquad (8)$$

Here a_{H} refers to the activity of the hydrogen ion, m_{H} to its molality, and γ_{H} to its individual activity coefficient. Formerly equation (8) was written with ion concentrations in place of

activities, and in this form it is known as the Nernst[5] formula (1889) for a concentration cell. Experimentally the positive pole of such a cell is the electrode in the solution of greater hydrogen ion activity. The polarity of the cell must be considered in using equation (8); if E is written with a positive sign, the single primes must refer to the more acid solution.

The use of equation (8) implies the existence of standard solutions of known hydrogen ion activity. One such solution which is available in any laboratory is 0.1 M hydrochloric acid. The best value for the activity coefficient of the hydrogen ion in this solution is 0.841, a figure which is due to Scatchard (104). For the method by which this figure was obtained, reference must be made to that author's paper. He concluded that the liquid junction potentials between saturated potassium chloride and dilute hydrochloric acid solutions (up to 0.1 M) were constant. It is really on an extension of this conclusion to all solutions in general that pH measurements are based. If his value of γ_H for 0.1 M hydrochloric acid is accepted, the pH of that solution is 1.075, and for a cell with two hydrogen electrodes, one being in the standard acid, equation (8) takes the form

$$\mathrm{pH} = 1.075 + \frac{E}{0.0001984T}. \qquad (9)$$

In using equation (9), E is written with a positive sign if the electrode in the standard acid is positive, and with a negative sign if that electrode is negative.

Many who have worked with hydrogen electrodes have preferred to follow Sørensen in basing pH values on a definite figure for the E.M.F. of a tenth-normal calomel cell in terms of the normal hydrogen electrode. This calomel cell consists of an electrode of mercury in contact with a 0.1 N potassium chloride solution saturated with calomel, Hg_2Cl_2. It is a constant and

[5] Hermann Walther Nernst (1864–), formerly Professor of Physical Chemistry at the University of Berlin, ranks with Arrhenius, van't Hoff, and Ostwald as one of the great figures of the science. He is particularly well known for his applications of thermodynamics to chemistry, and for his textbook, which has passed through many editions in various languages.

reproducible half cell, but any measurement with it involves a liquid junction with a more concentrated or saturated solution of potassium chloride. To avoid this junction between two potassium chloride solutions of different concentrations, many workers use a calomel cell made up with saturated potassium chloride. Any calomel cell must have a pole potential difference expressible in terms of the molal hydrogen electrode, which is a hypothetical half cell consisting of a hydrogen electrode saturated with hydrogen at 1 atmosphere pressure and dipping into solution in which the activity of hydrogen ions is exactly unity. Such a solution is not easily defined or prepared, and indeed this can be done only after a long series of measurements with very dilute solutions of a strong acid, such as were described by Scatchard in the paper mentioned. The adoption of any calomel cell as a standard in pH work involves the acceptance of some such series of measurements, usually that of Sørensen, and the assumption that the calomel cell in use is identical with that used by Sørensen. The reproducibility of the hydrogen electrode in 0.1 *M* HCl is probably as great as that of any calomel cell; hence it is recommended that a calomel cell should simply be a working standard, to be standardized each day against a hydrogen electrode. If a calomel cell is used in this way, the value of E to be used in equation (9) in calculating the pH of an unknown solution is the difference between the two E.M.F. values obtained with the same calomel cell against the hydrogen electrode in the unknown and in the standard HCl. Since a calomel cell is more positive than a hydrogen electrode in any possible aqueous solution, this difference E in equation (9) is positive if the E.M.F. observed with the calomel cell is greater in the case of the unknown solution than when the standard is measured.

Another useful standard solution of known pH is the "standard acetate" of Michaelis,[6] which consists of a solution 0.1 *N* with respect both to acetic acid and sodium acetate. It may be

[6] Leonor Michaelis (1875–), formerly of Berlin, now at the Rockefeller Institute for Medical Research in New York, is one of the most productive of living workers in the field of biochemical applications of physical chemistry.

prepared by mixing equal volumes of 0.4 *N* acetic acid and 0.2 *N* sodium hydroxide solutions. Its pH value is 4.62, which is in agreement with measurements based on the HCl standard already mentioned. If the standard acetate is used experimentally instead of 0.1 *M* HCl, equation (9) must of course be modified by using 4.62 instead of 1.075, keeping the same convention as to the sign of *E*.

Some Practical Details of pH Measurements. Hydrogen electrodes have been prepared in various shapes and sizes, the basis

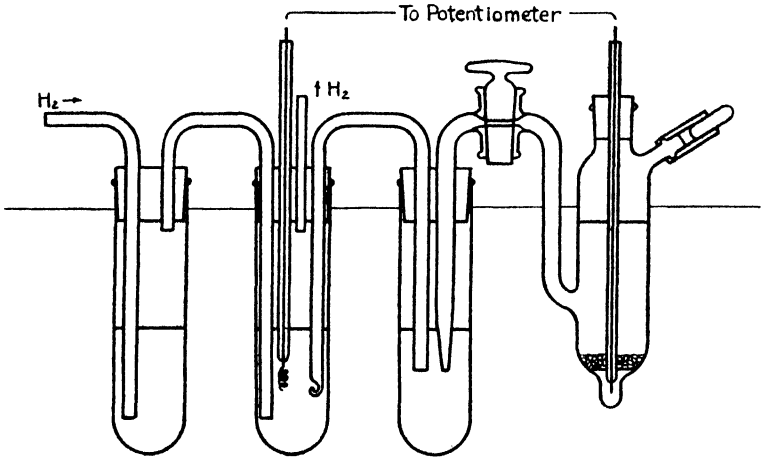

FIG. 10. Hydrogen and calomel electrodes.

being usually platinum foil or wire. One convenient form is that depicted in Fig. 10. It consists of a piece of thin platinum wire sealed into the end of a soft glass tube and bent into a coil before being platinized (coated with platinum black). Commercial tank hydrogen is bubbled through water in the tube on the left and then through the solution in the second tube, which is the hydrogen electrode vessel. Contact is made with saturated potassium chloride solution in the third tube by means of an agar bridge or siphon tube filled with a 3 per cent jelly of agar-agar made up in saturated potassium chloride solution. The end of the bridge tube in the hydrogen electrode vessel is drawn out and bent up to minimize diffusion, and the bridge is allowed to

touch the solution only when the E.M.F. is being measured. Such a bridge may be used repeatedly if kept in saturated potassium chloride solution when not in use. The vessel on the right is the calomel cell, contact being made with the mercury in the bottom by a short platinum wire sealed through the end of a glass tube. Practically the calomel half cell is more positive than the hydrogen electrode in a solution of any possible pH, so that the former is always connected to the positive and the latter to the negative wire of the potentiometer circuit. The outfit shown in Fig. 10 may be kept at constant temperature by immersion in a water thermostat, and it has the advantage of being made of simple parts.

For solutions in which foaming is excessive, the bubbling of hydrogen through the solution may give trouble. For such solutions the vessel of Clark ((26), p. 293) is recommended. He fills the empty vessel with hydrogen, displaces some of the gas by the solution, and establishes equilibrium by rocking the vessel so that the electrode is alternately exposed to solution and to hydrogen. His vessel has two special stopcocks, and its temperature is controlled by an air bath.

A convenient type of vessel using bubbling hydrogen is that of Simms (108), in which the hydrogen and calomel cells have double glass walls, so that the temperature may be controlled by circulating water from a thermostat between the walls.

For ordinary pH measurements, commercial electrolytic hydrogen gives good results without purification other than bubbling through water to saturate it with water vapor. Strictly, of course, it should be saturated with water at the partial pressure existing in the solution to be measured. Standardization of the electrodes with a solution of known pH at the beginning and end of a series of measurements makes it unnecessary to correct the E.M.F. for changes in the barometric pressure or for the partial pressure of water vapor. Such correction to one atmosphere of dry hydrogen can be made by the use of tables given by Clark, and the correction is of course necessary if it is desired to compare E.M.F. values rather than pH values.

The Measurement of Electromotive Force. This is done by the Poggendorf compensation or potentiometer method, in which a calibrated resistance is so adjusted that a fraction of the E.M.F. of a working battery is made exactly equal and opposite to the unknown E.M.F. of the cell. The E.M.F. from the working battery is checked by frequent calibration against a standard cell of known E.M.F. The diagram in Fig. 11 shows the elements of the wiring of a potentiometer circuit. AC is a uniform wire through which current flows from the battery B. This

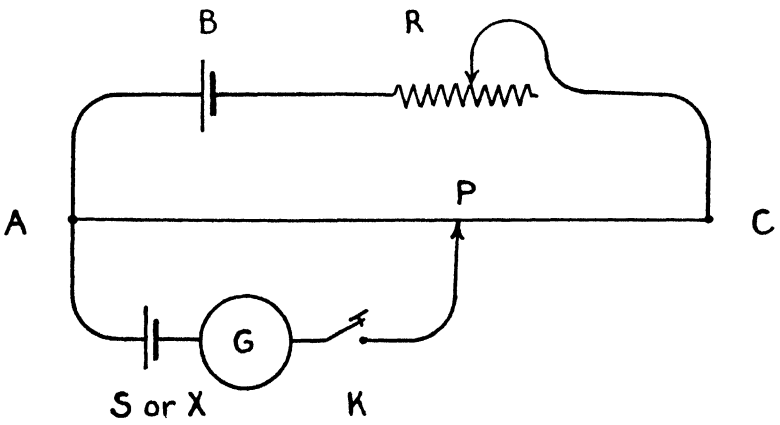

FIG. 11. Diagram of a potentiometer circuit.

current is adjusted by the rheostat R to a definite value such that the instrument gives the correct reading for the standard cell S when the key K is tapped and no current flows through the galvanometer G. After the current has been correctly adjusted, the cell to be measured, X, is connected in place of S, and a similar balance is obtained by moving P until no current flows through G when K is closed for an instant. Under these conditions the E.M.F. of the cell X is to the length of wire AP as the E.M.F. from the battery between A and C is to the length AC. Most potentiometers are provided with scales giving E.M.F. readings directly in volts. In using any potentiometer the connections must be so made that like poles of the battery and the

standard cell, or of the battery and the unknown cell, are connected to the same end of the wire AC.

For rough measurements the potentiometer and standard cell may be replaced by a rheostat and voltmeter, as indicated by Hildebrand (61).[7] For most pH measurements the E.M.F. need be read only to the nearest half or quarter of a millivolt, which is easily possible with a potentiometer graduated to one-half or one millivolt. For more refined measurements, such as those of cells without liquid junction, a standard potentiometer graduated to 0.05 millivolt is necessary if it is desired to take full advantage of the constancy and reproducibility of such cells.

The Quinhydrone Electrode. In solutions more acid than about pH 8, measurements of pH may be made by using an electrode of an inert metal such as gold or platinum simply dipping into the solution to which a few crystals of solid quinhydrone have previously been added. This substance is an equimolecular addition compound of quinone, $C_6H_4O_2$, and hydroquinone, $C_6H_4(OH)_2$, and it has a low solubility in aqueous solutions. In a concentration cell with two quinhydrone electrodes, the solutions being connected by a potassium chloride bridge, the E.M.F. varies with (H^+) according to the same formula that applies to the hydrogen electrode, equation (8). The calculation of pH from such measurements involves the use of a standard of known pH, just as in the case of the hydrogen electrode. The quinhydrone electrode has the practical advantages that it comes to equilibrium very quickly and that it gives constant potentials in solutions where the hydrogen electrode fails because of chemical action. An example is a solution of picric acid, $C_6H_2OH(NO_2)_3$, which is reduced by hydrogen in the presence of platinum black, giving drifting potentials, while it is unaffected by quinhydrone. Another advantage of the quinhydrone electrode in biological work is that the absence of a stream of gas avoids any loss of dissolved carbon dioxide, with consequent changes in pH. For example, the electrometric determination of pH in blood serum (34) is much simpler with

[7] This paper is particularly recommended for its clear exposition of the varied applications of hydrogen electrode measurements.

the quinhydrone than with the hydrogen electrode, although some drifts in potential have been noted. Loss of carbon dioxide is avoided by keeping the serum under a layer of inert mineral oil.

The quinhydrone electrode has the disadvantages that it does not work in alkaline solutions, its potential varies with the salt content of the solution much more than does that of the hydrogen electrode, and it gives drifting potentials with solutions containing certain proteins, such as gelatin.

The action of the quinhydrone electrode is explained by means of the theory of oxidation-reduction potentials, which is discussed in a later chapter.

The Glass Electrode. Very thin membranes of certain kinds of glass, if interposed between two solutions of varied (H^+), appear to act like hydrogen electrodes. The E.M.F. across the glass may be measured between two calomel half cells, each connected to one of the solutions on the two sides of the glass by a saturated potassium chloride bridge. A better arrangement is to keep a silver-silver chloride electrode in 0.1 *M* HCl on one side of the glass, with the standard or unknown solution on the other side, connected by a single KCl junction to a calomel electrode or other constant half cell. In this case the E.M.F. to be used in calculating pH is the difference between the E.M.F. readings obtained with the standard and unknown solutions. For many solutions of varied (H^+), the E.M.F. so obtained has been found to be related to the hydrogen ion activities by the Nernst formula, equation (8). Such measurements involve the practical difficulty that the high resistance of the glass makes it necessary to use an electrostatic instrument such as a quadrant electrometer in balancing the potentiometer circuit to measure the E.M.F. Several workers have been able to avoid the difficulties connected with the use of a quadrant electrometer by using a galvanometer with vacuum tube amplification (36). The preparation of glass electrodes of a convenient form has been described by MacInnes (79), who has also shown that the glass and hydrogen electrodes agree within 0.0002 volt in solutions from pH 2 to 8. These results were obtained only

after correction for an asymmetry potential which was found to exist when both sides of the glass were in contact with identical solutions. It seems likely that the glass electrode will find a wide application in biological studies on account of the absence of chemical reactions with the material under investigation; only glass and the salt bridge need be brought into contact with the fluid.

PROBLEMS

1. With two hydrogen electrodes and a saturated potassium chloride bridge, the pH of an unknown solution was measured against a standard buffer of pH 4.62. The E.M.F. was 0.153 volts at 20°C., the electrode in the standard solution being positive. Calculate the pH of the unknown solution. What would its pH have been if the polarity of the cell had been reversed?

2. A hydrogen electrode in 0.1 *M* hydrochloric acid was connected by a saturated potassium chloride bridge to a saturated potassium chloride calomel cell at 25°C. The E.M.F. was 0.3095 volts, the calomel electrode being positive. Calculate the E.M.F. of this calomel cell against a normal hydrogen electrode. How could this calculated value be used in getting a pH value from the E.M.F. obtained with this calomel cell against a hydrogen electrode in any unknown solution?

3. A quinhydrone electrode in an unknown solution was found to be 0.186 volt more negative than a similar quinhydrone electrode in 0.1 *M* HCl at 25°C. Calculate the pH of the unknown solution.

4. A concentration cell at 20°C., with zinc electrodes in two solutions connected by a potassium chloride bridge, gave an E.M.F. of 0.172 volt. The positive electrode was that in a standard solution in which the activity of Zn^{++} was 0.1. Calculate the activity of Zn^{++} in the other solution.

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CHAPTER VII

Adsorption; The Colloidal State

Adsorption. This term may be defined as a local change in concentration at an interface. Willard Gibbs[1] (45), in 1876, deduced from the laws of thermodynamics an important relation between surface tension and adsorption. Expressed qualitatively, the Gibbs principle states that if any component of a system has the property of lowering the surface tension at any interface in the system, the concentration of that component must be greater, if equilibrium exists, in the interface than in adjacent parts of the system. Conversely, a material which raises surface tension must be less concentrated in the surface. The mathematical relation derived by Gibbs between the excess concentration in the interface, the concentration in the rest of the system, and the rate of change of surface tension with concentration has been experimentally verified in a few cases of adsorption at the interface between two immiscible liquids. In most cases of adsorption that have been studied it has not been possible to test or use this relation because one phase of the system is usually a solid, and nothing very definite is known about the interfacial tension between a solid and a gaseous or liquid phase.

[1] Josiah Willard Gibbs (1839–1903), Professor of Mathematical Physics in Yale University, was one of the foremost of scientific thinkers. His paper "On the Equilibrium of Heterogeneous Substances" (45) contains many of the fundamental principles of physical chemistry, deduced by abstract mathematical reasoning from the laws of thermodynamics. Chemists associate his name primarily with the phase rule, the Gibbs-Helmholtz equation for the temperature coefficient of the electromotive force of a reversible cell, and this law of adsorption. A number of other important principles, later re-discovered independently by others, seem to be implied in some of the equations of Gibbs; among these are van't Hoff's equation for the osmotic pressure of dilute solutions, Nernst's formula for the electromotive force of a concentration cell, and Donnan's equation for membrane equilibrium. His contribution to pure mathematics seems, moreover, to have been of comparable importance to his work in the field of thermodynamics.

The material which has been most used in studies of adsorption is charcoal, which has the property of taking up on its surface considerable quantities of gases or dissolved substances which may be brought into contact with it. It is, of course, this property which led to the use of charcoal in gas masks in the war of 1914–18.

In adsorption experiments equilibrium is usually reached within a few minutes, and the existence of a real equilibrium condition may sometimes be proved by approaching it from both directions. For example, if a given mass of charcoal is found to adsorb the same amount of gas at a given pressure when this pressure is reached experimentally either from a higher or a lower pressure, then equilibrium may be said to exist. Similar experiments have been made in the case of adsorption from solution, by approaching a given concentration either directly or by dilution. In some cases, however, the velocity of adsorption is so much greater than that of the reverse process that the complete removal of an adsorbed material from a solid surface by washing with the original solvent may be a very slow if not a humanly impossible process. In such cases it is usually possible to hasten the removal by using another solvent in which the point of equilibrium is very different, or often, in the case of aqueous solutions, by changing the pH or chemical composition of the solution. Such a reversal of the process of adsorption from solution has been termed elution.

If an adsorption equilibrium is studied at constant temperature, with varied pressure (in the case of a gas) or concentration (in the case of a solution), and the amounts of substance adsorbed are plotted against the equilibrium pressures or concentrations, the resulting curve is called an adsorption isotherm. Such curves may often be represented, over a limited range, by an empirical parabolic equation,

$$q = ap^m. \tag{1}$$

Here q is the quantity of substance adsorbed by a given mass of adsorbent, p the equilibrium pressure or concentration, and a and m are constants. The exponent m is usually a fraction be-

tween 0.2 and 1. Equation (1) is often called Freundlich's[2] adsorption isotherm (44). Its applicability to a given set of data may easily be tested by plotting logarithms of the values of the two variables; if the points fall on a straight line, the equation fits, and the values of the constants a and m are readily obtained from the intercept and slope of the line. It is to be emphasized that this equation has no theoretical basis, and is merely a convenient interpolation formula which fits some data. Experimentally it is usually found that the amount adsorbed at high concentrations or pressures becomes constant. Such a saturation or limiting value is not given by equation (1), according to which the values of q should always continue to rise as p is increased.

Other equations or isotherms have been proposed by various workers, but there is no single equation which fits all types of adsorption. One other may be mentioned which was deduced by Langmuir[3] (74) for the case of the adsorption of a single layer of gas molecules on a plane surface. This may be written

$$q = \frac{Ap}{B + p} \tag{2}$$

where q is the quantity adsorbed, p the equilibrium pressure or concentration, and A and B are constants. This hyperbolic equation predicts that, when p becomes very large, q will approach a limiting value, A. It may be tested by plotting p/q against p. If the equation fits the data, the points will fall on a straight line, and the values of the constants A and B may be obtained from the slope and intercept of the line. Fig. 12 shows the difference in the shape of the graphs of the Freundlich and Lang-

[2] Herbert Freundlich (1880–), of the Kaiser Wilhelm Institute for Physical Chemistry, Berlin, is one of the well known colloid chemists of the present day. His book (44) is the most authoritative work on surface effects and colloid chemistry.

[3] Irving Langmuir (1881–), of the Research Laboratory of the General Electric Co., Schenectady, N. Y., is a brilliant contemporary physical chemist. Working from the viewpoint of pure science, in order to satisfy intellectual curiosity, he has obtained results which have often been of great industrial value. His study of the behavior of gases in contact with hot wires led to the development of the gas-filled electric light bulb.

muir equations, when they are made to coincide at two points by a proper selection of the constants. Langmuir (74) found that his equation applied to the adsorption of various gases on the surface of mica or glass, and it has also been found (64) to apply to the removal of proteins from solution by collodion membranes. It has been pointed out (65) that an identical equation may be derived from the law of mass action as applied to a reversible reaction between two substances in solution, the total

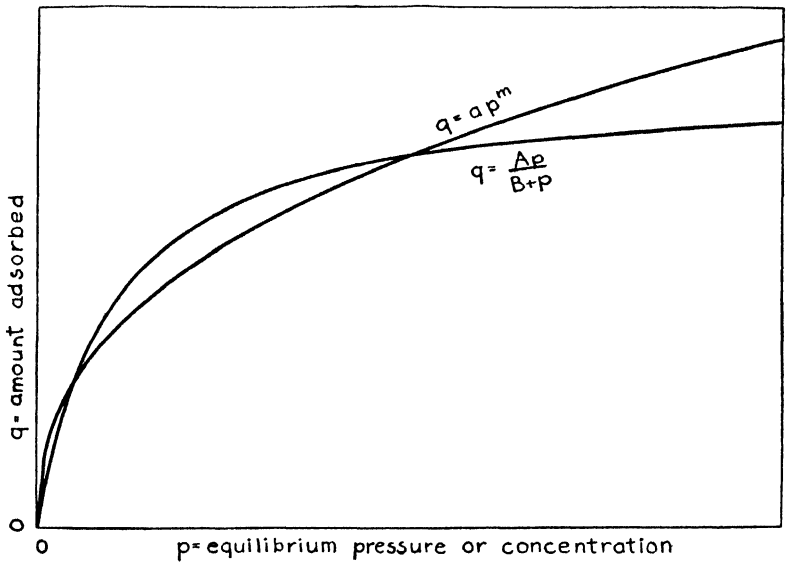

FIG. 12. Adsorption isotherms.

concentration of one being kept constant while that of the other is varied. The fact that this equation may fit certain data obtained with colloidal solutions is of no use in deciding whether the reaction studied is adsorption or chemical combination. Langmuir was careful to point out that "no single equation other than purely thermodynamic ones should be expected to cover all cases of adsorption. . . . "

The extent of adsorption in general decreases with increase in temperature, as does the surface tension of liquids.

Dispersed Systems. Ordinarily gold is considered to be insoluble in water. Yet if gold is dispersed or scattered in water in

the form of extremely fine particles, as may be done by passing an electric arc between two gold wires under water, the result is a fairly stable solution containing little else but gold and water. It differs from a true solution in that the gold may be separated from the solvent by the application of very strong centrifugal force, or by filtration under pressure through certain membranes. This colloidal solution of gold is an example of a dispersed system, which may be defined as a mixture of at least two kinds of matter, one of which is in the form of very fine particles. The fine particles are often called the dispersed phase, the discontinuous phase, or the internal phase (corresponding to the solute in the case of true solutions), while the material in which the particles are scattered may be called the dispersion medium, the external phase, or the continuous phase (corresponding to the solvent in the case of true solutions). Dispersed systems are known in which the continuous phase may be solid, liquid or gaseous, and similar variations are possible in the dispersed phase. Those which have been most studied, however, are the colloidal solutions, especially those in which the dispersion medium or solvent is water or an aqueous solution.

Colloidal Solutions. Dispersions of the types solid in liquid and liquid in liquid include coarse suspensions such as sand in water and emulsions such as oil in water, which separate spontaneously into two phases on standing under the influence of gravity. They also include, at the other extreme, true solutions such as salt in water and alcohol in water. Between these two extremes lie colloidal solutions, which differ from suspensions and emulsions in having smaller particles, and differ from true solutions in having larger particles. The divisions between these classes are not rigid, but as an approximation it may be stated that the particles in true solutions are less than 1 mμ[4] in diameter, that colloidal particles range from 1 to 100 mμ, and particles larger than 100 mμ are included in suspensions and emulsions.

Colloidal particles are further characterized by their slow rate of diffusion and their inability to pass through membranes of parchment paper, gold beater's skin, or collodion. The latter

[4] Millimicron, 10^{-7} cm., sometimes written $\mu\mu$. A micron, μ, is 10^{-4} cm.

property is the basis of the process known as dialysis, whereby a colloidal solution is freed from electrolytes by enclosing it in such a membrane which is dipped into pure water. Ultrafiltration is another process in which this property is used; here a colloidal solution is filtered under pressure through such a membrane, the larger particles being retained by the filter while molecules in true solution as well as the solvent or dispersion medium may pass through. The fact that certain particles do not pass through the membrane, however, does not necessarily mean that the membrane has pores smaller than the particles; it is possible that the particles may be retained by surface forces or adsorption on the membrane, even though its pores may be considerably larger than the particles.

Another characteristic of colloidal solutions is the property of light scattering known as the Tyndall[5] effect. A familiar example of this is the visibility of a beam of light when viewed from the side in a darkened room, due to reflection by dust particles in the air. Similarly if a powerful beam of light is passed through a colloidal solution and viewed at right angles to the path of the light, the path appears as a luminous band, while a similar experiment with a true solution shows only darkness. The Tyndall effect is the basis of the ultramicroscope,[6] which has been much used in the study of colloids. Here light is directed horizontally through a layer of the solution, which is observed from above with an ordinary microscope. In the case of certain colloids, as gold, the individual particles produce intensely luminous dots, by means of which the particles may be counted and their motion observed. These luminous dots are not true optical images of the particles, but are simply the result of the scattering of light by them. Hence the size of such particles cannot be

[5] John Tyndall (1820-1893), Professor of Natural Philosophy at the Royal Institution, London, was known for researches in various branches of physics, especially heat, and as a popular lecturer and writer of textbooks on scientific subjects. He was particularly interested in Alpine glaciers.

[6] This instrument was devised in 1903 by Henry Siedentopf (1872–), of the optical works of Carl Zeiss, Jena, and Richard Zsigmondy (1865–1929), who was later Professor of Inorganic Chemistry at the University of Göttingen, Germany.

obtained by direct observation with an ultramicroscope. The name of this instrument implies that particles too small to be seen with an ordinary microscope may be detected by it. Most colloidal solutions of biological origin, however, show very little in the ultramicroscope. Although a gross Tyndall effect may be seen, the individual particles do not show up as bright spots, probably because their index of refraction is very close to that of the solvent.

Brownian Movement. The motion of colloidal particles was mentioned above. This is not limited to such small particles, but may be observed with an ordinary microscope in many relatively coarse suspensions. Its discoverer, a botanist named Brown, first noticed it with pollen grains (1828). With colloidal gold in the ultramicroscope this motion appears as a violent and rapid zigzag movement of each individual particle. The smaller the particles, the more rapid is the motion. It is explained by the kinetic theory as due to the momentary unequal bombardment of each particle by the true molecules of the suspension medium around it. Since these molecules are so numerous, the resultant force of all their bombardments on a large particle would be zero; hence the motion appears only with particles small enough to receive unequal impacts from the molecules around it. A rather striking example of Brownian movement of the particles in living protoplasm may be obtained by observing an ameba with a dark field microscope.

Classification of Colloids. The term colloid was formerly applied to certain substances or kinds of matter such as starch, proteins and glue, in contrast to the term crystalloid, which was applied to substances forming crystalline solids and true solutions. Since some proteins have been crystallized, and such crystalloids as sodium chloride have been obtained in colloidal solution in appropriate solvents, the terms colloid and crystalloid are now used only to refer to substances which are in the colloidal state or the crystalloidal state, the distinction being largely one of particle size. Colloidal solutions are often called sols; a sol which has become semi-solid or jelly-like is called a gel.

Colloidal solutions are broadly divided into two classes. The first class, variously known as suspensoids, lyophobic colloids, or irreversible colloids, includes most of the inorganic sols, such as colloidal gold, platinum, silver and arsenic sulphide. Such sols resemble suspensions in consisting of solid particles dispersed in a liquid; they show but little attraction between the solvent and the particles, as they are easily precipitated by low concentrations of electrolytes; after evaporation to dryness, they do not spontaneously go back into colloidal solution when treated with fresh solvent. Moreover their viscosity differs very little from that of the solvent.

The second class is called the emulsoids, lyophilic sols, or reversible colloids. Sols of this class were originally supposed to be like emulsions in belonging to the liquid in liquid type of systems. There seems to be some doubt about this resemblance, so that it is better to replace the term emulsoids by lyophilic colloids. This implies an attraction between the two phases, which is inferred from the fact that high concentrations of salts are required to precipitate such sols. After evaporation to dryness, these colloids are reversible or spontaneous in that the sol is usually re-formed when fresh solvent is added. This class includes most sols of biological origin, such as starches, albumins, gelatin, etc. Those who believe the two phases are liquid assume that the sol consists of particles of concentrated solution of the colloidal solid dispersed in a more dilute solution. These sols are regarded by others as composed of swollen, hydrated particles of solid dispersed in the solvent. The viscosity of such sols is usually much greater than that of the solvent.

Electrical Properties of Colloidal Solutions. Substances in colloidal solution are found usually to migrate to one pole or the other if an electric current is passed through a sol. Such migration is called cataphoresis. From measurement of the velocity of this motion in a known electrical field, it is possible to calculate, according to Helmholtz,[7] the value of the potential differ-

[7] Hermann Ludwig Ferdinand von Helmholtz (1821–1894) began his remarkable scientific career as a military surgeon. After holding chairs of physiology in several German universities, he became Professor of Physics

ence which must exist between the particle and the solution. This electrokinetic potential, or at least the migration velocity from which it is calculated, is considerably influenced by the addition of various electrolytes to a sol. Many colloids have a charge of fixed sign, either positive or negative. Some, like the proteins, are amphoteric, changing the sign of their charge as (H^+) or (OH^-) is increased. For each protein some value of (H^+) is found at which neither positive nor negative charge predominates; this is its isoelectric point. In the case of some colloids it is possible to replace a negative charge by a positive, not only by increasing (H^+), but by increasing the concentration of some other positive ion such as La^{+++}. In many cases a close connection seems to exist between the size of the electrokinetic potential and the stability of the sol; if added electrolytes reduce the potential below a certain limiting value, the sol becomes unstable and the dispersed phase is precipitated.

Colloidal Complexes. It has not been possible to prepare stable gold sols absolutely free from electrolytes. Indeed, a definite small trace of electrolyte seems to be necessary to ensure stability, although slightly higher concentrations cause precipitation. Similar results have been obtained with ferric oxide. Such experiments have led to the complex theory of colloids, according to which an insoluble and inert particle is made stable, and given an electric charge, by combination with one type of ion of the electrolyte present. This combination with an ion, whether it be truly chemical or due to adsorption, probably explains the origin of the charge of most colloids. Precipitation of such sols is supposed to occur when the added ions are such as to combine with the ion in which the charge originates. In such precipitation a marked effect of valence is found, ions of high valence being effective precipitants at very low concentrations. Comparative experiments have indicated that the effec-

at Berlin, and finally President of the Physikalisch-Technische Reichsanstalt at Charlottenburg, an institution of somewhat the same nature as our Bureau of Standards at Washington. His physiological work covered many branches of the science, particularly the sensations of sight and hearing. He is probably best known for his fundamental work in pure physics, especially in electricity and thermodynamics.

tive precipitating ion and the particle precipitated have charges of opposite sign.

Protective Colloids. A lyophobic colloid such as a gold sol may be protected against precipitation by small concentrations of electrolyte if it is previously treated with a hydrophilic colloid such as a gelatin sol. The latter appears to form a surface film on the gold particles, so that the surface exposed to the solution behaves like gelatin and not like colloidal gold. Colloidal gold has been used in testing various lyophilic colloids, the "gold number" of an emulsoid being an inverse measure of its ability to protect a gold sol against precipitation by NaCl solution. Tests based on this protective effect have been used in the diagnosis of diseases affecting the colloids of the spinal fluid.

Lyotropic or Hofmeister Ion Series. In the early days of colloid chemistry, Hofmeister (1888) studied the effect of various salts on the precipitation (salting-out) of the proteins of egg white and on the swelling of blocks of gelatin jelly. By comparing series of salts in which the anion was varied but the cation kept the same, and vice-versa, he found it possible to arrange the individual ions in series with regard to their effect on the property studied. He obtained such series as citrate > tartrate > $SO_4^=$ > acetate > Cl^- > NO_3^- > I^- > CNS^- for anions, and $Li^+ > K^+ > Na^+ > NH_4^+ > Mg^{++}$ for cations, in the case of the salting out of egg white from neutral solutions. Similar studies of other properties or other lyophilic colloids gave somewhat similar series, although under certain conditions the order was reversed. These series seemed to have no particular relation to the chemical nature of the ions, and it was believed that they were characteristic of colloidal phenomena. Other workers, however, found similar series in studying properties of systems not in the colloidal state, as in the effect of salts on the surface tension of water and on the hydrogen electrode potentials in solutions of HCl or H_2SO_4 containing added salt. The effects responsible for such series are now believed to be concerned with the attraction of the salts for water, and hence they are not primarily colloidal characteristics. Freundlich has proposed for

such effects the term lyotropic, indicating a change in the solvent. In the work of Loeb on the swelling of gelatin, he found that such ion series were absent or inconspicuous if the comparison was made at the same pH. The concentrations of salt used by him, however, were much less than those of Hofmeister. Michaelis found, in agar and konyaku, other colloidal jellies, that at low salt concentrations the swelling was influenced mainly by the pH of the medium and the valence of the added ions, in agreement with Loeb, while at high salt concentrations he found pronounced lyotropic series, in agreement with Hofmeister.

Theories of Colloidal Phenomena. It is obvious from geometrical considerations that a given mass of a substance such as gold, in the finely divided colloidal condition, has a tremendous extent of surface, as compared to that of the same mass in a single lump. Such considerations have led many to believe that colloidal behavior is best explained as due to surface action or adsorption. Yet there is evidence that some solutions which show colloidal behavior may consist simply of immense individual molecules dispersed in water, and the surface of a molecule in solution is not ordinarily considered to be the seat of adsorption or surface energy. Accordingly other chemists have attempted to explain colloidal behavior as far as possible on the basis of chemical reactions rather than surface effects. Most of the fundamental problems of colloidal chemistry are far from settled at the present time.

Surface Films. In addition to the study of dispersed particles, colloid chemistry includes also the study of thin films at surfaces. Starting with the idea that surface phenomena may be referred to forces essentially chemical in nature, Langmuir (73) and Harkins[8] (53) developed the concept of a layer of oriented molecules at a surface. Langmuir studied the spreading of films composed of a fat, or one of the higher fatty acids, on the sur-

[8] William Draper Harkins (1873–) is Professor of Chemistry in the University of Chicago. He has done extensive experimental and theoretical work in the field of surface chemistry.

face of water. He assumed that, when a continuous film occupied the greatest possible area, the film was only one molecule thick, and that the molecules of the fatty substances were oriented with their carboxyl groups towards the water. On this basis he calculated the dimensions (length and cross-section) of single molecules. The results are in agreement with values obtained by other methods (69), and the existence of surface layers of oriented molecules is regarded as well established. Langmuir also measured, by a special type of balance, the horizontal force exerted by these films on a paper strip in the plane of the surface. Such measurements have yielded considerable information about the physics and chemistry of surfaces. Work in this field is described in the books of Adam (3) and Rideal (101). Films of protein materials have been studied by Du Noüy (38) and Gorter (48).

PROBLEMS

1. By plotting the following data on the adsorption of ammonia gas by charcoal, show whether the equation of Freundlich or that of Langmuir applies:

Pressure at equilibrium, cm. Hg.	30.9	76.8	139.3	195.3	244.7	290.7
Amount of gas adsorbed per g. charcoal, cc.	8.4	14.3	21.0	26.3	30.7	33.9

2. Treat as in Problem 1 the following data on the adsorption of nitrogen on mica:

Pressure, dynes per cm.^{2}	2.8	4.0	6.1	13.0	34.0
Amount adsorbed, mm.^{3}	12.0	15.1	19.0	25.5	33.0

3. Calculate the number of atoms in a spherical particle of gold 3 mμ in diameter. (Atomic weight = 197.2, density = 19.3, Avogadro's number = 6.06×10^{23}.)

4. Compare the surface of 1 g. of gold in particles 3 mμ in diameter with the surface of the same mass of gold in a single sphere.

5. If a solution of serum albumin containing 1.07 g. in 100 ml. has an osmotic pressure of 2.69 mm. Hg at 1°C., what is the

molecular weight of the serum albumin? If 1 g. of this protein occupies 0.745 cc., and its molecules are spherical, what is the diameter of a molecule?

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CHAPTER VIII

Membrane Equilibrium

In studying the colloidal behavior of proteins, Loeb[1] (76) showed that many observations could be explained by the application of Donnan's[2] (35) theory of membrane equilibrium. Since this theory has found other applications of physiological importance, it will be given in brief outline at this point.

Distribution of Diffusible Ions across a Membrane. Donnan deduced his theory for a system composed of two compartments, each containing an aqueous solution, but separated by a membrane of a peculiar semi-permeability. The membrane must be freely permeable to the solvent and to most dissolved substances, including electrolytes, but absolutely impermeable to at least one species of ion present in solution. This non-diffusible ion species is in one compartment only, and is kept there by the impermeability of the membrane to it. Water and any diffusible ions, being able to penetrate the membrane, will tend to be distributed, as a result of diffusion, between the two compartments in such a way as to approach equilibrium. Suppose the electrolytes present to be hydrochloric acid and an ionized chloride, RCl, the membrane being impermeable to the ion R^+. The ions H^+ and Cl^- can penetrate the membrane, but they must do so in pairs, not separately, because of the electrostatic forces between them. The probability that a hydrogen ion and a

[1] Jacques Loeb (1859–1924) was one of the most brilliant and versatile of biological scientists. Born and educated in Germany, he came to this country to teach at Bryn Mawr College. He did most of his scientific work at the Universities of Chicago and California, the Rockefeller Institute in New York, and the Marine Biological Laboratory, Woods Hole, Massachusetts. He made important and original contributions in such diverse fields as the physiology of the brain, tropisms, regeneration in plants, antagonistic salt action, artificial parthenogenesis, and the colloidal behavior of proteins. The guiding principle in all his work was the attempt to explain biological facts on a mechanistic basis.

[2] Frederick George Donnan (1870–) is Professor of Physical Chemistry at University College, London.

chloride ion will arrive simultaneously at a given point on the membrane depends on their concentrations in the solution, being directly proportional to the product of the concentrations of these two ion species.[3] The rate of diffusion of hydrochloric acid through the membrane in one direction must therefore be proportional to the product of the concentrations of its ions in the compartment from which it is diffusing, and the same is true for its diffusion through the membrane in the reverse direction. At equilibrium these two rates of diffusion must be equal. The equilibrium condition therefore implies an equality of the products of the concentrations, in the two compartments, of the two ions of a diffusible electrolyte.

z	R^+	
y	H^+	$H^+\ x$
$y+z$	Cl^-	$Cl^-\ x$

In the diagram the vertical line represents the membrane, and the ions are distributed between the two phases or compartments as indicated by their chemical symbols. If the small letters represent the concentrations of the ions at equilibrium, the relation deduced by Donnan is expressed by the equation

$$x^2 = y(y+z). \tag{1}$$

It will be observed that this kinetic derivation does not make use of the presence of the non-diffusible ion. The equation is still true for a system containing no such ion, for in this case $z=0$ and $x=y$, which means that the diffusible ions are equally distributed.

For the case where a non-diffusible ion is present, equation (1) implies that $x>y$. Since the product of two equal numbers, x, is equal to the product of two unequal numbers, y and $y+z$, x is the geometric mean of y and $y+z$. Its value therefore lie,

[3] The probability that a single ion will hit a given point on the membrane is obviously dependent on the concentration of that ion species. The probability that two independent events will happen simultaneously is equal, according to the laws of probability, to the product of their individual probabilities.

between those of $y + z$ and y, and since all these numbers are positive, x must be greater than y. This means that although the ions H^+ and Cl^- can pass freely through the membrane, their concentrations on the two sides at equilibrium are not equal if a non-diffusible ion is present on one side. It is this unequal distribution of diffusible ions at equilibrium which is the outstanding feature of the Donnan equilibrium.

Donnan's derivation of the equality of ion products was made by means of thermodynamics; hence the equation is strictly exact only in terms of the activities of the diffusible ions. Ion concentrations are used here for simplicity in formulating the equations.

If the valence of the non-diffusible ion is more than 1, equation (1) still holds if z is taken to refer to the equivalent concentration or normality of the non-diffusible ion instead of its molar concentration. If the valence of any diffusible ion is more than one, the equation must be altered. For example, if the system contained R_2SO_4 and H_2SO_4, the relation would be

$$x^3 = y^2(y + z). \tag{2}$$

This is a consequence of the fact that every $SO_4^=$ ion that passes the membrane must be accompanied by two H^+ ions, so that the relation of equality of ion products involves the product of $(SO_4^=)$ and $(H^+)^2$.

Effect of Added Electrolytes. Equation (1) may be put into the form

$$\frac{x^2}{y} = y + z, \text{ or } \frac{x^2}{y^2} = \frac{y + z}{y}, \text{ or } \frac{x}{y} = \sqrt{1 + \frac{z}{y}}. \tag{3}$$

If hydrochloric acid is added to the system, the values of x and y will obviously have to increase. If the concentration of the non-diffusible ion is unchanged, z is constant. Any increase in y must, therefore, according to equation (3), result in a decrease in the value of the ion ratio x/y. If y increases without limit, z being constant, the value of the ratio approaches 1. This means that the addition of a diffusible electrolyte, in this case having an ion in common with the non-diffusible electrolyte, tends to diminish or abolish the unequal distribution of diffusible ions.

The same effect may be produced by the addition of any diffusible electrolyte, irrespective of whether it has an ion in common with the non-diffusible electrolyte. For example, consider a system containing RCl, HCl, and $NaNO_3$. The ions will be distributed as indicated by the chemical symbols in the diagram, in which the small letters again refer to concentrations.

z	R^+		
$z+y+n-q$	Cl^-	Cl^-	$x+m-p$
y	H^+	H^+	x
n	Na^+	Na^+	m
q	NO_3^-	NO_3^-	p

The law of the equality of the ion products at equilibrium leads to the following equalities of ion ratios:

$$\frac{x}{y} = \frac{m}{n} = \frac{q}{p} = \frac{z+y+n-q}{x+m-p}. \tag{4}$$

An application of the algebraic rules of proportion to the last two members of equation (4) gives an additional equality,[4]

$$\frac{q}{p} = \frac{z+y+n}{x+m}. \tag{5}$$

Let λ represent the value of the ion ratio in equations (4) and (5). Then

$$\lambda = \frac{z+y+n}{x+m}. \tag{6}$$

Since $m = \lambda n$ and $x = \lambda y$, it follows from equation (6) that

$$\lambda = \frac{z+y+n}{\lambda y + \lambda n}, \text{ or } \lambda^2 = \frac{z+y+n}{y+n}, \text{ or } \lambda = \sqrt{1+\frac{z}{y+n}}. \tag{7}$$

This means that if the concentration of the non-diffusible ion is kept constant, while that of any other ion of like sign in the same solution is increased, the value of the ion ratio decreases and

[4] This may be derived from the last two members of equation (4) by clearing of fractions, cancelling the term $-pq$, and factoring out q and p again.

approaches 1. In other words, the addition to the system of a large amount of any diffusible electrolyte tends to abolish the unequal distribution of ions.

Membrane Potentials. If equilibrium exists in a system of the type considered by Donnan, it should not be possible to make the system do work. If such a system is converted into a galvanic cell by the introduction of electrodes reversible with respect to one species of diffusible ion, the electromotive force of the cell must be zero if there is true equilibrium. For example, if a hydrogen electrode or a silver-silver chloride electrode is dipped into each compartment of the system containing RCl and HCl, no E.M.F. should be found. This prediction has been verified.

This fact may seem at first sight to be at variance with the requirement of an unequal distribution of diffusible ions. If the activities of H^+ or Cl^- in the two solutions are not equal, there should be unequal electrode potentials at the two hydrogen or silver-silver chloride electrodes. This is also true. The answer to this apparent paradox is that there is in the system a third seat of potential difference; namely, between the two solutions on opposite sides of the membrane. Donnan predicted that such membrane potentials should exist, and that their magnitude should be given by an equation identical with the Nernst formula for a concentration cell with two hydrogen electrodes in solutions connected by a salt bridge, but that their sign should be opposite to that of a hydrogen electrode concentration cell. This prediction has been verified by Loeb, in numerous experiments in which a solution containing a protein and hydrochloric acid was on one side of the membrane, with aqueous hydrochloric acid alone on the other side. In these experiments the membrane potential was measured by the use of two identical calomel electrodes, made up in saturated potassium chloride solution (Fig. 13). Two liquid junctions were involved, one between each of the solutions of the equilibrium experiment and the saturated potassium chloride leading to the calomel electrode. The potassium chloride solutions, separating the calomel electrodes from the solutions in contact with the membranes,

prevented the electrodes from being in reversible equilibrium with any ion in the system; hence the thermodynamic requirement of zero E.M.F. did not exist. The calomel cells were simply used as equal and opposite half cells which constituted an inert means of leading off, for the purpose of measuring it, any potential difference already existing between the two solutions on opposite sides of the membrane. Such measurements give the true

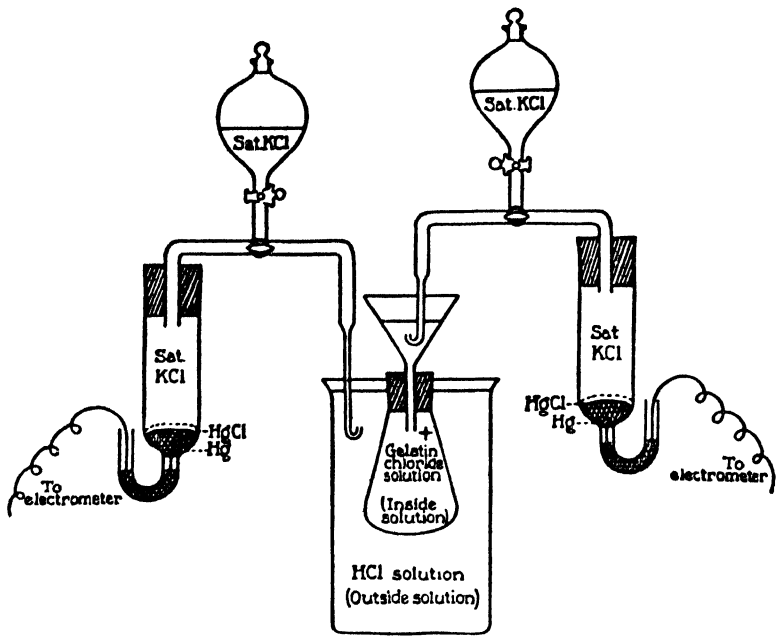

FIG. 13. Method of measuring membrane potentials. (Loeb)*

value of the membrane potential difference if it is justifiable to assume that the liquid junction potentials between the saturated potassium chloride and the two solutions of the system are equal and opposite. This assumption is the same as that used in all pH measurements. Similar assumptions are used in physiological studies of potential differences in muscles, nerves, and other tissues, where contact with the electrodes is usually made

* Reproduced, by permission of the publisher, from Jacques Loeb, *Proteins and the Theory of Colloidal Behavior*. McGraw-Hill Book Co., New York, 1922, 1924.

by means of physiological sodium chloride solution rather than potassium chloride, because of the toxic effect of the latter. It is possible that such bioelectric potential differences may be due, in part at least, to membrane potentials of the type predicted by Donnan and measured by Loeb.

Osmotic Pressure. For systems of the type under discussion, Donnan's theory predicts an inequality in the osmotic pressures of the two solutions at equilibrium. For dilute solutions van't Hoff's law (equation (13), Chapter III) may be assumed to hold for osmotic pressures. For the system containing RCl and HCl the osmotic pressure difference should then be given by the relation

$$\Pi = \Pi_1 - \Pi_2 = RT(2z + 2y - 2x). \tag{8}$$

If the valence of the non-diffusible ion is not 1 but n, its molar concentration is z/n, and the osmotic pressure relation is

$$\Pi = RT\left(\frac{z}{n} + z + 2y - 2x\right). \tag{9}$$

In this equation the osmotic pressure of the non-diffusible electrolyte depends on the terms containing z, while the observed pressure difference is less than this by an amount depending on the unequal distribution of diffusible electrolyte, or the difference between x and y. If x and y are exactly equal, which can be strictly true only when both are zero, the observed osmotic pressure difference would be

$$\Pi_c = RT\left(\frac{z}{n} + z\right) \tag{10}$$

which is the true osmotic pressure of the colloidal or non-diffusible electrolyte alone. This pressure could also be obtained from equation (9) if x, y and Π were measured. It should be possible to calculate n from such measurements, after eliminating z by equation (1).

If equation (9) is divided by equation (10), one obtains the ratio of the osmotic pressure difference observed in a Donnan membrane experiment to the true osmotic pressure of both ions

of the non-diffusible electrolyte. This ratio is

$$\frac{\Pi}{\Pi_c} = \frac{\frac{z}{n} + z + 2y - 2x}{\frac{z}{n} + z} = 1 - \frac{2(x-y)n}{z(n+1)}.$$

From equation (1),

$$z = \frac{x^2}{y} - y = \frac{x^2 - y^2}{y} = \frac{(x-y)(x+y)}{y}.$$

These two equations may be combined to give

$$\frac{\Pi}{\Pi_c} = 1 - \frac{2n}{n+1} \cdot \frac{y}{x+y}. \qquad (11)$$

If the value of y is very small, which is the case when very little diffusible electrolyte is present, equation (3) shows that the ratio x/y is much greater than 1, or x is much greater than y. Under these conditions the second term in equation (11) approaches zero, so that the ratio of the observed to the true osmotic pressure of the colloidal electrolyte, though always less than unity, approaches that figure as y is decreased. Only in the ideal case of a colloidal salt which was not at all hydrolyzed, with no added diffusible electrolyte, would the observed osmotic pressure be exactly equal to the true osmotic pressure, unless the membrane happened to be impermeable to both ions of the colloidal electrolyte.

Quite a different result is obtained in the presence of high concentrations of a diffusible electrolyte. In this case y is large, and according to equation (3), if z is constant while y is increased, the value of x/y becomes less and approaches 1. Accordingly the value of x, while always greater than y, approaches y as the value of the latter is increased. Under these conditions the factor $y/(x+y)$ in equation (11) approaches $\frac{1}{2}$ and the term to be subtracted from 1 approaches $n/(n+1)$. The ratio of the observed osmotic pressure to the true osmotic pressure of the colloidal electrolyte therefore approaches $1 - n/(n+1)$, which is

$1/(n+1)$. For example, if n, the valence of the non-diffusible ion, is 1, the observed osmotic pressure is only a little over half of the true osmotic pressure of the non-diffusible salt. If n is 100, the observed pressure becomes almost as low as $1/101$ of the true pressure. This rather striking relationship gives another method of calculating the valence of a non-diffusible ion (15).

Such osmotic pressure differences have been measured by Loeb and others by a very simple method. This consists in putting a solution of a colloidal or non-diffusible electrolyte such as a protein salt, either with or without the addition of a diffusible electrolyte, inside of a collodion sack closed by a rubber stopper carrying a vertical glass tube to act as a manometer (Fig. 5, p. 31). The membrane is submerged in a solution of the diffusible electrolyte in a beaker or flask, and after equilibrium is attained the apparent osmotic pressure is measured in terms of the difference in level between the solution in the manometer tube and that in the outer vessel. By such measurements Loeb found that the effects of acids and bases on the apparent osmotic pressure of protein solutions were due to changes in the unequal distribution of diffusible ions. The changes were due in part to alterations in the concentration of non-diffusible ions (z in equation (1)) produced by the combination of protein with H^+ or OH^- from the added acid or base, and in part to the depressing effect of higher concentrations of diffusible electrolyte. He found no evidence that the observed effects were due to any colloidal phenomena other than the non-diffusibility of the protein ions. In studies of the actual osmotic pressure of proteins, as distinct from the apparent pressure in the presence of diffusible electrolytes, it is necessary either to make a correction for the Donnan effect, to have the concentration of diffusible electrolytes so high that it is negligible, or to work at the isoelectric point of the protein (109, 2). In the latter case there should be no unequal distribution of ions because there is no excess of either positive or negative non-diffusible ions; neutral *Zwitterionen* should not cause a Donnan effect.

Colloidal Behavior of Proteins. The Donnan theory was shown by Loeb (76) to account quantitatively for the pecu-

liar effect of added acid or alkali on the membrane potentials and the apparent osmotic pressures obtained with protein solutions. If the protein was at its isoelectric point, the values of these properties were at a minimum. If a diffusible acid was

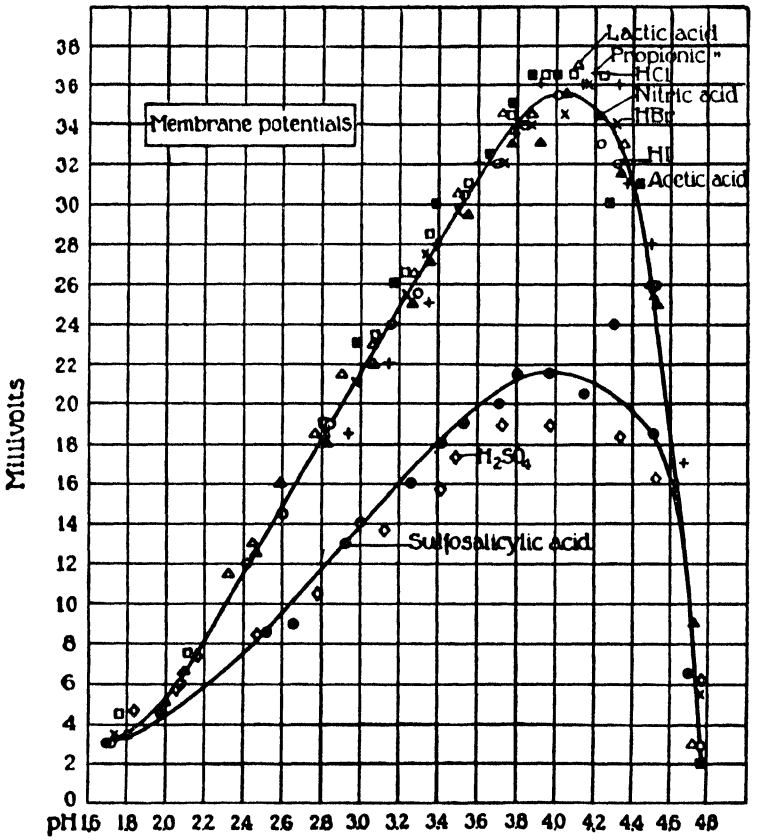

FIG. 14. Membrane potentials of 1% gelatin in solutions of mono-basic and di-basic acids. (Loeb and Kunitz)*

added, the measured values increased, due to the formation of non-diffusible ions by the union of H^+ with protein. Still further increase in acid concentration caused the values to fall again, as eventually all the protein became ionized or combined with H^+,

* J. Loeb and M. Kunitz, *J. Gen. Physiol.*, 5, 671 (1922–23).

and the excess acid exerted a depressing effect on the Donnan ratio, as has already been pointed out. The general shape of the curves obtained in such experiments, in which the protein concentration was constant and the amount of diffusible acid present was varied, is illustrated by Figs. 14 and 15. When either

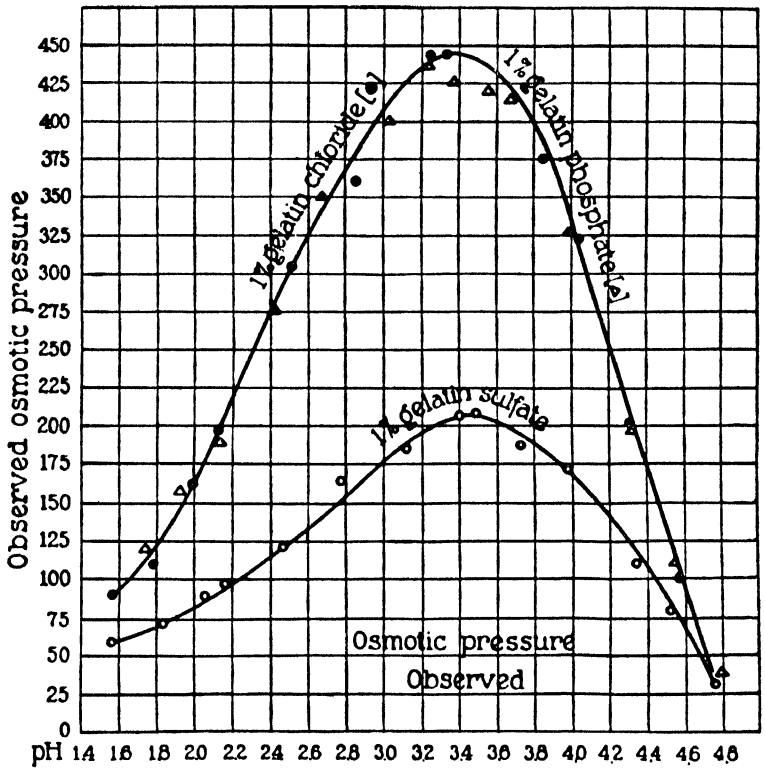

FIG. 15. Apparent osmotic pressures of 1% gelatin in solutions of mono-basic and di-basic acids. (Loeb)*

the membrane potentials or the osmotic pressures are plotted against pH, the curve obtained has the form of a hump, high in the middle and low at both ends. Similar curves have been obtained with other proteins, such as egg albumin, casein, edestin, and serum globulin. In these experiments with protein and acid the sign of the membrane potential was such that the protein

* J. Loeb, *J. Gen. Physiol.*, 3, 692 (1920–21).

solution was always positive. In experiments in which alkali was added instead of acid, the general results were of the same nature, but the sign of the membrane potential was reversed, the protein solution being negative, as the theory predicts for the case of a non-diffusible anion.

Loeb showed that it was possible to reproduce the experimental curves for the membrane potentials almost exactly by values calculated from the Nernst equation and the measured pH values of the inside and outside solutions. While this agreement does not prove quite so much as Loeb thought it did, it does show that the two solutions were in equilibrium, even though their ion activities were widely different. He further showed that curves quite similar to the osmotic pressure curves could be calculated from the measured pH values by combining equations (1) and (9), neglecting altogether the term z/n, which is reasonable if the valence of the protein ion is very high. If Π is expressed in millimeters of water, the value of RT for 24°C. is about 2.5×10^5. The relation used is therefore

$$\begin{aligned} \Pi &= 2.5 \times 10^5 (z + 2y - 2x). \\ &= 2.5 \times 10^5 \left(\frac{x^2}{y} + y - 2x \right) \\ &= 2.5 \times 10^5 \frac{(x - y)^2}{y}. \end{aligned} \tag{12}$$

The values of Π so obtained differed somewhat from those observed. Part of this difference is due to the form of equation (12), since a small error in either of the hydrogen ion concentrations may cause a large error in the square of their difference. This may be shown by using the measured values of Π and y, and solving the equation for x, which gives

$$x = y + \frac{\sqrt{y\Pi}}{500}. \tag{13}$$

When equation (13) is applied to Loeb's data, the calculated values for $-\log x$, the pH of the outside solution, are found to agree fairly well with those observed. This may be taken as

evidence that nearly all of the apparent osmotic pressure observed in such experiments is due to the unequal distribution of diffusible ions which is predicted by Donnan's theory.

Following an idea of Procter and Wilson, Loeb applied the Donnan theory to explain the effects of pH on the swelling of particles of solid gelatin in acid. At low temperatures gelatin does not dissolve appreciably in aqueous solutions, but imbibes water or swells and becomes jelly-like. In Loeb's experiments equal weights of powdered or granular gelatin were allowed to swell in acid solutions of varied concentration, and the amount of swelling was determined by filtering off the moist swollen particles and weighing them. The swollen particles were then melted or dissolved by warming, and the pH values of the resulting solutions were measured. When the amount of swelling was plotted against pH, curves were obtained which closely resembled those for the apparent osmotic pressure of gelatin in acid solutions as measured in collodion bags, the high and low points of the two sets of curves falling almost exactly at the same pH. This result was explained by assuming that H^+ ions may be rendered non-diffusible by combining with gelatin in the gel state. This restraint on the diffusibility of certain ions sets up a Donnan equilibrium even though there is no semi-permeable membrane other than the framework of the jelly, which is penetrated by water and diffusible ions. Diffusible ions distribute themselves unequally between the particles of jelly and the solution around them, producing a higher osmotic pressure inside the particles. This results in the entrance of water, causing the particles to swell. This osmotic swelling is resisted by the elasticity of the jelly, so that a condition of approximate equilibrium is reached in which the osmotic force is just balanced by the elastic forces in the jelly network. The extent of the swelling is a measure of the difference in osmotic pressure between the solution in the jelly particles and the external solution. Hence the theory predicts the similarity observed between the effects of pH on the apparent osmotic pressure and on the swelling.

Curves of similar shape were obtained by Loeb in studying the viscosity of gelatin solutions containing acid, and he ex-

plained these, too, by an application of Donnan's theory. Following Einstein,[5] he assumed the viscosity to depend on the fraction of the volume of the solution occupied by dissolved or colloidal particles. He further assumed that solutions of gelatin contained invisible submicroscopic particles capable of swelling, and that this hypothetical swelling was affected by pH in the same way as the gross measurable swelling of blocks or granules of jelly. If the existence of such particles is granted, the explanation of the viscosity curves follows at once from that of the swelling curves. Loeb applied this explanation only to solutions of gelatin, which have a much higher viscosity than solutions of other proteins under comparable conditions. In the case of egg albumin solutions, which have a low viscosity, the effect of pH on the viscosity is much less striking; hence Loeb believed that the hypothetical particles were absent, but that the dissolved protein was molecularly dispersed.

Loeb further proposed that colloidal behavior should be defined, in the case of proteins, to include only those phenomena which depend on the existence of non-diffusible ions and the consequent unequal distribution of crystalloidal ions. This definition is not accepted by certain other workers in this field, prominent among whom is Pauli.[6] He explains the effects of varied acidity on many properties of protein solutions, including their electrical conductivity and viscosity, as due to parallel variations in the hydration and the extent of ionization, or activity of the ions, of protein-acid compounds. Other workers maintain that there is no connection between hydration and ionization in protein solutions (128).

Although Loeb's definition of colloidal behavior and his explanation of viscosity changes have not been universally ac-

[5] The name of Albert Einstein (1879–) is familiar to all, as he is one of the greatest of theoretical physicists. Not so familiar, perhaps, is his early work, in which he applied his mathematical genius to some of the problems of physical chemistry, such as specific heats, viscosity, osmotic pressure and diffusion.

[6] Wolfgang Pauli (1869–) is Director of the Institute for Medical Colloid Chemistry of the University of Vienna, Austria. His work on the colloid chemistry of proteins is summarized in a book (98) which gives a thorough review of the literature of this subject.

cepted, there seems to be little doubt that his application of Donnan's theory to proteins was a great step in the clarification of colloid chemistry, and that his work in this field is a good foundation for the future explanation of many things of physiological importance.

PROBLEMS

1. The membrane potential of a 1 per cent solution of purified egg albumin containing hydrochloric acid was found to be 0.0185 volt at 24°C., the outside solution, which contained no protein, being electrically negative. The pH value of the outside solution was 2.91. Calculate the pH of the inside solution (the observed value was 3.24).

2. Using the observed value for the pH of both solutions in Problem 1, calculate the osmotic pressure difference between the two solutions, assuming the protein molecules and ions to have a negligible osmotic pressure, and expressing the result in millimeters of water (the observed value was 218 mm. H_2O).

3. Using the observed value for the osmotic pressure difference in Problem 2 and the observed value for the pH of the inside solution in Problem 1, calculate the pH of the outside solution.

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CHAPTER IX

Equilibria in Blood

A complete study of the blood and and of other physiological fluids does not, of course, belong in the field of pure physical chemistry. Yet so much progress has been made in explaining the behavior of blood by means of physical chemistry that a brief consideration of some of the facts and explanations may be of interest at this point. Much of the experimental work has been done with horse blood, but human blood and the blood of other mammals seem to behave in much the same way.

Constituents of Blood. The amount of blood in the human body is about 8 per cent of its total weight. Every one is familiar with the general appearance of blood; it is about 5 times as viscous as water and about 5 per cent heavier (specific gravity = 1.05). Microscopic examination shows that the color of blood is due to the red cells or erythrocytes, which look pale yellow under the microscope. The blood count, or number of red cells per cubic millimeter of blood, averages about 5,000,000. These red cells are bi-concave disks about 8.6μ in diameter and 2.6μ thick. It requires but little calculation to convince one of the tremendous surface of the red cells, which makes possible an extremely rapid diffusion of dissolved substances in either direction between the cells and the surrounding liquid. The blood contains also smaller numbers of white cells or leucocytes (about 1 leucocyte to every 500 erythrocytes), as well as very small cells called platelets (about 350,000 per cu. mm.) which are believed to be concerned with the coagulation or clotting of blood.

The red blood cells contain about 35 per cent of protein, chiefly oxyhemoglobin, about 1 per cent of other electrolytes (about 0.12 M), and a fraction of a per cent of non-electrolytes. Somewhat less than 65 per cent of the cell contents is water. Any treatment of the cells which causes the coloring matter, oxyhemoglobin, to come out of the cells is called hemolysis. This may occur as a result either of putting the cells into water or any aqueous solution much more dilute than plasma, of repeated

ing and thawing of the blood, or of the addition of any substance which destroys the cell membranes, such as ether, saponin, bile salts, or certain other organic substances.

The liquid in which the blood cells are suspended is called the blood plasma. It may be separated from the cells by centrifuging. The dissolved substances in the plasma consist of proteins (about 8 per cent), other electrolytes (about 1 per cent, or 0.16 *M*), and non-electrolytes (about 1 per cent). The plasma is thus a fairly dilute aqueous solution, being about 90 per cent water. Of the plasma proteins, about 70 per cent is albumin, 25 per cent globulin, and about 5 per cent fibrinogen. The clotting of blood or plasma consists mainly in the transformation of the soluble protein fibrinogen into the insoluble protein fibrin. In this process a jelly is formed which then contracts spontaneously, expressing a fluid which is the blood serum. Serum has approximately the same composition as plasma, except for the absence of fibrinogen. Clotting may be prevented by the addition of any substance which removes calcium ions (potassium oxalate or sodium citrate or fluoride is often used for this purpose) or by the addition of certain materials of animal origin such as heparin, which is obtained from liver, hirudin, from leeches, or snake venoms. Because of the necessity of adding a foreign substance to plasma in order to keep it from clotting, much of the experimental work on the equilibria between blood cells and the surrounding medium has been done with serum rather than plasma. For this purpose the blood is usually defibrinated before clotting occurs. This may be done by vigorous stirring or whipping, or by shaking it with glass beads; the fibrin then clings to the rod or beads as it is formed, and does not form a continuous jelly throughout the whole mass of blood. In this way the corpuscles are left suspended in serum instead of being caught in the meshes of the clot and removed from the serum.

While the inorganic ions in blood are present in rather low concentrations, the total being not more than 0.16 *M*, their presence is of considerable importance for the normal functioning of the blood. Aside from ionized proteins, the chief ions present

are Na^+, K^+, Ca^{++}, Mg^{++}, Cl^-, HCO_3^-, $H_2PO_4^-$, $HPO_4^=$, $SO_4^=$, and, perhaps least in amount although of extreme importance, the ever-present H^+ and OH^-.

Gases in Blood. Blood contains in solution the common gases of air, oxygen, nitrogen, and carbon dioxide. The chief function of the blood is the transportation of oxygen to, and the removal of carbon dioxide from, the other tissues of the body. It is in the explanation of the mechanism of this respiratory function of the blood that physical chemistry has, up to the present time, been of most use to the science of physiology.

The solubilities of oxygen and carbon dioxide in blood are peculiar in two respects. In the first place, blood is capable of carrying in solution much more of these gases than is water, or any solution of simple electrolytes, under comparable conditions. Second, the solubility of these gases in blood appears not to follow Henry's law (Chapter II). For example, the solubility of oxygen, at a pressure of 1 atmosphere, in water at the normal temperature of blood, 37°C., is given as 2.39 volumes per cent. In alveolar air (air obtained from the lungs by a forced expiration at the end of a normal expiration) the partial pressure of oxygen is about 100 mm. Hg; accordingly, by Henry's law, the solubility of oxygen at this pressure in water at 37° would be $2.39 \times 100 \div 760 = 0.314$ volumes per cent. Actually the total solubility of oxygen in blood is much greater than this, being of the order of 20 volumes per cent. This figure represents a limiting or saturation value which is attained when the partial pressure of oxygen in equilibrium with the blood is about 100 mm. or higher. As the oxygen pressure is decreased below 100 mm., the amount of oxygen held by the blood drops along a curve which is concave towards the pressure axis (Fig. 16).

The results obtained on equilibrating carbon dioxide with blood are qualitatively of the same nature, except that no limiting or saturation value is reached. Again, however, the curve of the amount dissolved as a function of the partial pressure is concave towards the pressure axis, and exhibits a marked deviation from the straight line corresponding to Henry's law. Fig. 16 shows, on the same scale, the total solubilities, in volumes per

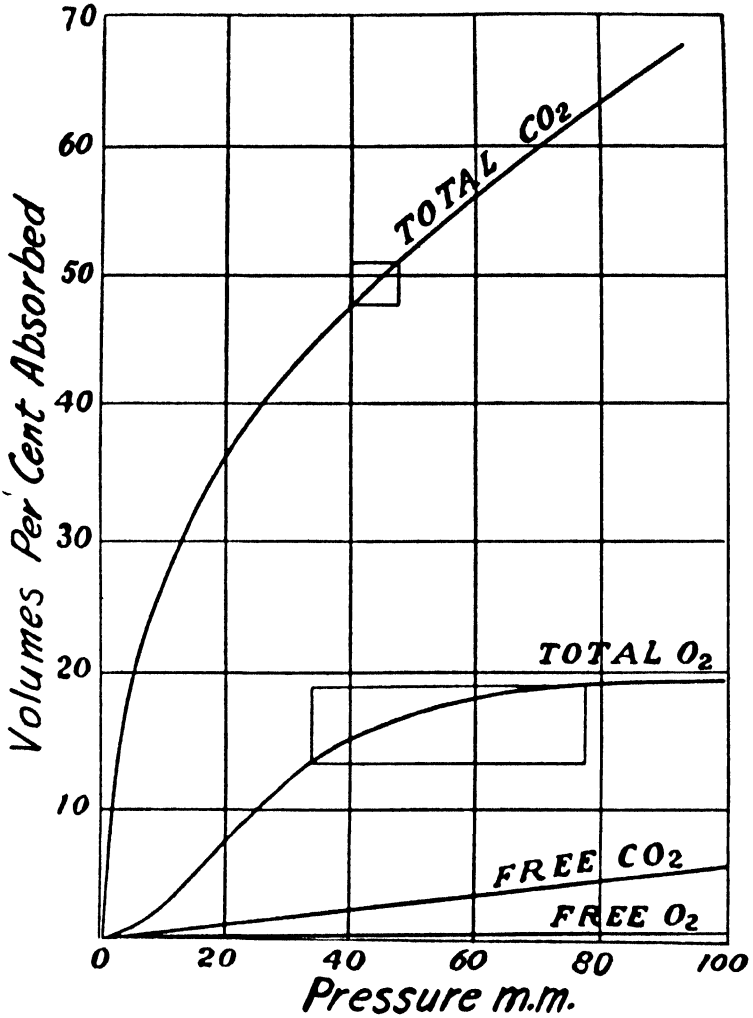

FIG. 16. Solubility curves of oxygen and carbon dioxide in blood. (L. J. Henderson)*

* Reproduced, by permission of the publisher, from L. J. Henderson, *Blood: a Study in General Physiology*. Yale University Press, New Haven, 1928.

cent, of oxygen and of carbon dioxide in a sample of human blood, as well as the estimated amounts of the free or uncombined gases dissolved under similar conditions. It will be noticed that these two lower curves are straight lines, in agreement with Henry's law.

In the case of oxygen, the excess solubility represented by the difference between the upper and lower curves for oxygen in Fig. 16 is due to the presence of the red cells. This follows from the fact that the solubility of oxygen in blood plasma is much less than in whole blood, being about the same as in water, and following Henry's law. The solubility of oxygen in solutions of purified hemoglobin is represented by curves qualitatively similar to the solubility curve of oxygen in whole blood.[1] These curves, too, become horizontal at high pressures of oxygen, indicating a maximum capacity of hemoglobin to take up oxygen. The maximum number of moles of oxygen so taken up is quite exactly equivalent to the number of gram-atomic weights of iron present in the hemoglobin. The combining weight so obtained was about 16,700 grams of hemoglobin for each mole (32 g.) of oxygen, while the containing weight for each gram-atom (55.84 g.) of iron was the same. A similar figure was obtained for the weight of hemoglobin which is capable of combining with 1 mole of carbon monoxide. The figure 16,700 is now believed to represent the equivalent or combining weight of hemoglobin rather than its molecular weight, since a value of about 4 times this figure has been obtained for the molecular weight by the method of osmotic pressure (2) (68,000 ± 2,000), by the use of the ultra-centrifuge (117) (66,700 to 70,900), and by a method based on rate of diffusion (93) (68,600 ± 1,000).

The carriage of oxygen by the blood is generally explained as due to its reversible chemical combination with hemoglobin. The changes in the oxygen content of blood during respiration are referred to the immediate response of this reversible reac-

[1] The combination of hemoglobin with oxygen has been extensively studied by Joseph Barcroft (1872–), Professor of Physiology in Cambridge University, England. His book (10), which is written in an unusually informal and attractive literary style, may be heartily recommended to students.

tion to changes in the partial pressure of oxygen in the air in the lungs and in the tissue fluids with which the blood comes into contact.

In the case of carbon dioxide, the explanation is not quite so simple. Blood plasma is capable of taking up this gas, the curve obtained being of the same general shape as the upper curve for carbon dioxide in Fig. 16. Since blood is known to contain bicarbonate ion, it might be thought that its capacity for taking up carbon dioxide were due to the reaction

$$CO_3^{=} + H_2CO_3 \rightleftarrows 2HCO_3^{-}.$$

The first dissociation constant of carbonic acid, under the conditions prevailing in blood, is about 7.9×10^{-7} ($pK_1 = 6.10$), and the second dissociation constant is only about 1/10,000 as large. An application of the theory of buffer action shows that the concentration of the ion $CO_3^{=}$ which could exist at pH 7.4, the the normal value for blood, must be very low, and that even less could exist at the lower pH values produced by adding more CO_2 than the blood normally contains. By using the values just given for pH and pK_1 in the Henderson[2]-Hasselbalch[3] equation (Chapter V, equation (5)) it may be calculated that the normal ratio of free CO_2 (the sum of the dissolved CO_2 and H_2CO_3) to combined CO_2 (HCO_3^{-}) is about 1:20. The greater part of the carbon dioxide in blood is therefore in the form of bicarbonate ion, and the absorption or loss of this gas by the blood must involve the following equilibria:

$$CO_2 + H_2O \rightleftarrows H_2CO_3 \rightleftarrows H^+ + HCO_3^{-}.$$

An increase in the partial pressure of carbon dioxide in equilibrium with blood should therefore increase the acidity. One of the peculiarities of blood is that such changes in pH are much less than might be expected.

[2] Lawrence Joseph Henderson (1878–), Professor of Biological Chemistry in Harvard University, was one of the first (1908) to formulate the theory of buffer action in terms of the law of mass action and to apply it to the equilibria in blood.

[3] K. A. Hasselbalch (1874–), of Copenhagen, Denmark, was among the first (1912) to apply the hydrogen electrode to the measurement of pH in blood.

Another peculiarity is that all of the carbon dioxide in blood can be removed by exposing it to a vacuum, while from a simple aqueous solution of sodium bicarbonate only half of the total carbon dioxide can be so removed.

$$2NaHCO_3 \rightleftarrows Na_2CO_3 + H_2O + CO_2.$$

The removal of the remaining carbon dioxide can, of course, be accomplished by adding an acid stronger than carbonic acid. Blood plasma behaves like a sodium bicarbonate solution in that not all of its carbon dioxide can be removed by a vacuum, while more can be obtained by acidification. This indicates that the blood corpuscles contain something which has the same effect as acidification with respect to the liberation of carbon dioxide. It has been found that the addition of red blood cells to previously evacuated plasma results in the evolution of carbon dioxide on further evacuation.

Reciprocal Influence of Oxygen and Carbon Dioxide in Blood. If samples of the same blood (or hemoglobin solution) are exposed to mixtures of gases containing both oxygen and carbon dioxide at different partial pressures, it is found that the presence of each gas has an effect on the amount of the other which is absorbed. Fig. 17 shows the effect of varied partial pressures of carbon dioxide on the total solubility of oxygen in the blood of a certain man.[4] The abscissae represent partial pressures of oxygen in equilibrium with the blood and the ordinates are the amounts of oxygen in the blood, expressed as percentages of the maximum amount which this blood could absorb. The numbers on the four curves are the partial pressures of carbon dioxide in the gas mixtures. It is evident that an increase in the partial pressure of carbon dioxide materially decreases the amount of oxygen absorbed at any given partial pressure of oxygen. This

[4] This effect was discovered by Bohr, Hasselbalch and Krogh in 1907. Christian Bohr (1855–1911), Professor of Physiology in the University of Copenhagen, was the father of Niels Bohr, mathematical physicist and winner of a Nobel prize. August Krogh (1874–), now Professor of Animal Physiology in the same university, is one of the leading physiologists of the present day.

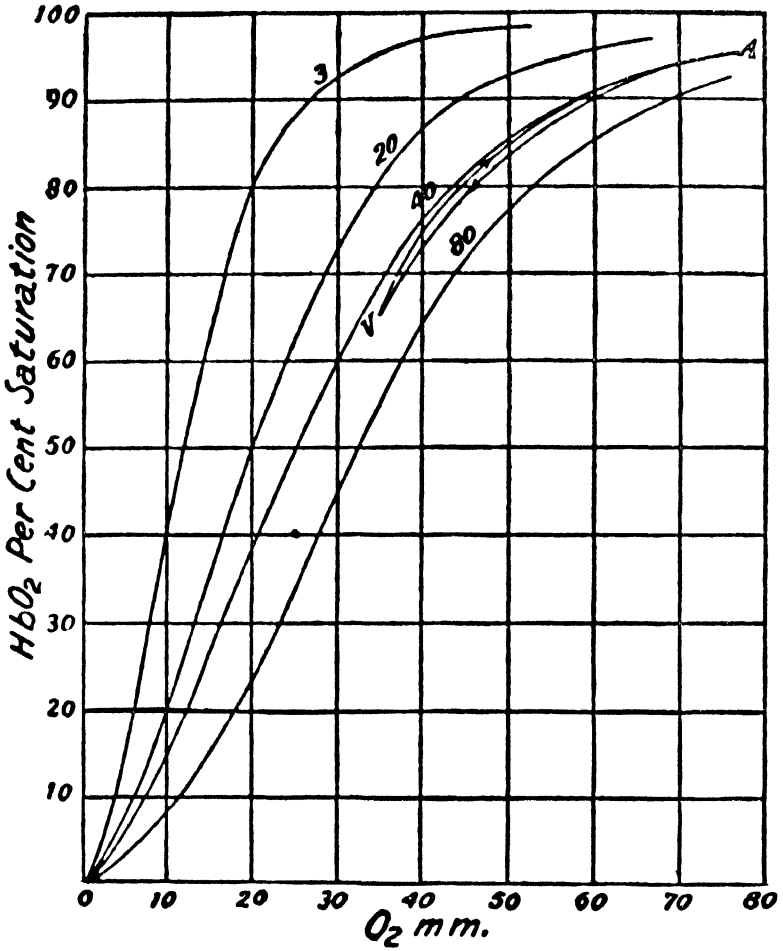

FIG. 17. Effect of carbon dioxide on the solubility of oxygen in blood. (L. J. Henderson)*

effect of carbon dioxide seems to be due to acidity, since other acids have a similar effect.

The reciprocal effect of oxygen pressure on the absorption of carbon dioxide is illustrated by Fig. 18, in which the lower curve, marked *O*, was obtained with fully oxygenated blood, and the

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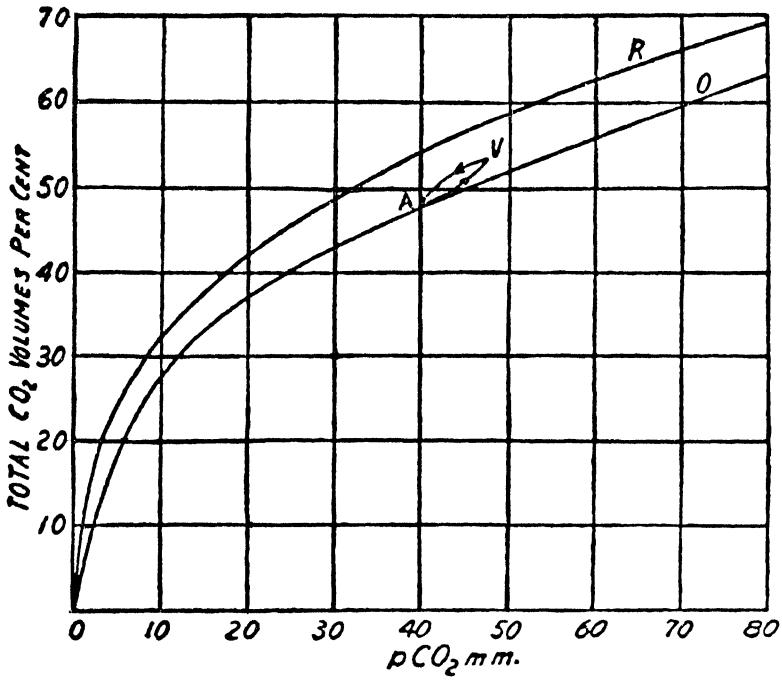

FIG. 18. Effect of oxygen on the solubility of carbon dioxide in blood. (L. J. Henderson)*

upper curve, marked *R*, with blood completely reduced or deprived of oxygen. It will be noted that a decrease in the oxygen pressure was accompanied by an increase in the amount of carbon dioxide taken up at any given partial pressure of the latter gas.[5]

These effects have been explained by Van Slyke's[6] work on the

* Reproduced, by permission of the publisher, from L. J. Henderson, *Blood: a Study in General Physiology*. Yale University Press, New Haven, 1928.

[5] This effect of oxygen pressure on the carbon dioxide capacity of blood was discovered by Christiansen, Douglas, and Haldane in 1914. John Scott Haldane (1860–), of Oxford, England, has been for years one of the foremost physiological workers in the field of respiration. His son, J. B. S. Haldane, is also widely known for work in biochemistry and for his popular essays on scientific subjects.

[6] Donald Dexter Van Slyke (1883–), Member of the Rockefeller Insitute for Medical Research, New York City, is one of the most active experimental workers in the field of blood chemistry, and is widely known for the quantitative methods which he has introduced.

base-binding capacities of oxyhemoglobin and reduced hemoglobin (99, 120). Both of these substances are proteins, and accordingly may behave as amphoteric electrolytes. In blood they behave only as acids, because any possible pH value of the blood is greater than that of either of their isoelectric points, which are given as 6.65 and 6.8 respectively. On the alkaline side of this isoelectric region, over a range of pH including all physiological values, it has been found that oxyhemoglobin is always combined with more alkali than is reduced hemoglobin, if the comparison is made at the same pH. Similarly if the same amount of base is added to equally concentrated solutions of the purified proteins, the solution containing the oxygenated hemoglobin is always found to be more acid. These facts have confirmed the idea that oxyhemoglobin is a stronger acid than reduced hemoglobin; that is, the molecule of oxyhemoglobin contains at least one acidic group whose dissociation constant is greater than that of the corresponding group in the molecule of reduced hemoglobin.

This assumption is the basis of the explanation of the reciprocal effect of the two gases. The addition of oxygen forms a stronger acid. The hydrogen ions so produced by the ionization of oxyhemoglobin are at once taken up by the buffer action of the bicarbonates to form carbonic acid, which breaks up to furnish an excess of carbon dioxide; hence the blood can no longer hold so much of the latter gas. If carbon dioxide is forced into the blood, the hydrogen ions so produced are at once taken up by the negative ions of oxyhemoglobin to form an excess of the undissociated molecules of this protein. Since the latter is in equilibrium with reduced hemoglobin and oxygen, this equilibrium is displaced with the liberation of oxygen, so that the blood can now hold less oxygen. This behavior may be indicated diagrammatically by the following series of interlocking equilibria, in which the formula HHb represents the molecule of reduced hemoglobin, and $HHbO_2$ that of oxyhemoglobin:

$$\begin{array}{ccccc} O_2 + HHb & \rightleftharpoons & HHbO_2 & \rightleftharpoons & H^+ + HbO_2^- \\ \uparrow\downarrow & & & & + \\ H^+ & & & & HCO_3^- \\ + & & & & \uparrow\downarrow \\ Hb^- & & & & H_2CO_3 \rightleftharpoons H_2O + CO_2. \end{array}$$

Both forms of hemoglobin may furnish hydrogen ions, but the ionization of carbonic acid in the diagram has been connected only with that of oxyhemoglobin because the latter is the stronger acid, and hence its ionization will have more effect on that of carbonic acid, and vice versa. An increase in oxygen pressure will evidently displace all the equilibria towards the right and downwards, while an increase in the pressure of carbon dioxide must have the reverse effect.

Distribution of Substances Between Cells and Plasma. One remarkable characteristic of red blood cells is that they are apparently impermeable to the ions of sodium and potassium. Most of the potassium of the blood is inside of the cells, and stays there under experimental as well as under normal conditions, while the greater part of the sodium remains in the plasma. Other ions, apparently, may pass from one phase to the other; chloride and bicarbonate, which are normally about twice as concentrated in the serum as in the cells, can be made to distribute themselves even more unequally by diminishing the pressure of carbon dioxide with which the blood is in equilibrium. Increasing the carbon dioxide pressure not only reverses this effect, but causes the cells to swell by osmosis. Moreover it has been found that hydrogen ions are somewhat more concentrated (or at least exhibit greater activity towards the hydrogen electrode) in the cells than in the serum. The cells appear to be permeable to hydrogen ions, as the distribution of these ions shifts with changes in the pressure of carbon dioxide. The cells show no evidence of permeability to proteins or to protein ions. The fact that the cells are permeable to some ions but not to others suggests that the conditions in blood are similar to those assumed by Donnan in deriving his equation for ionic distribution in a system containing a non-diffusible ion on one side of a membrane.

Donnan's equation was applied with some success to experiments on blood by Van Slyke (120). He assumed that the osmotic pressure of the solution within the cells was always equal to that of the serum or plasma outside. This assumption may be justified by freezing point measurements, and by the bi-concave

shape of the cells. If the cell contents had a greater osmotic pressure than the serum, water would flow in and make the cells spherical, as it does when the cells are put into a dilute salt solution. If the serum had a greater osmotic pressure, water would flow out of the cells, causing them to shrivel or become crenated. The assumption of equal osmotic pressures is not really inconsistent with the reasoning by which Donnan predicted an osmotic inequality in simple cases of membrane equilibrium, for in the blood there are non-diffusible ions on both sides of the membrane.

From analytical data Van Slyke calculated that most of the osmotic pressure in the cells and serum should be due to K^+, Na^+, Cl^-, and HCO_3^-, while of the proteins only hemoglobin is present in high enough concentration to be of much osmotic significance. It is to be noted that most of these osmotically effective ions are univalent. If Donnan's equation applies, the ratio of chloride ion concentration in the cells to that in the serum, which Van Slyke designated by the symbol r, must be equal to the corresponding ratio for bicarbonate ion concentrations or to the ratio of the sums of the concentrations of these two ions, or to the reciprocal of the corresponding ratio of the concentrations of hydrogen ion. If the Donnan theory applies,

$$r=\frac{(Cl^-)_c}{(Cl^-)_s}=\frac{(HCO_3^-)_c}{(HCO_3^-)_s}=\frac{(Cl^-)_c+(HCO_3^-)_c}{(Cl^-)_s+(HCO_3^-)_s}=\frac{(A^-)_c}{(A^-)_s}=\frac{(H^+)_s}{(H^+)_c}. \quad (1)$$

Here the subscripts c and s refer to cells and serum, the parentheses refer to concentrations (as an approximation for ionic activities), and (A^-) means the sum of the concentrations of all univalent diffusible negative ions. The ions Na^+ and K^+, to which the cells are impermeable, do not appear in the equation for ion ratios.

If the osmotic pressures of the cells and serum are equal, and if the difference in the osmotic pressures of the proteins in the two phases is assumed to be negligible as a first approximation, it follows that

$$(B^+)_c + (A^-)_c = (B^+)_s + (A^-)_s. \quad (2)$$

Here (B^+) refers to the sum of the concentrations of all osmotically effective univalent positive ions; that is, to $(Na^+) + (K^+)$. The concentrations are expressed as molalities, or moles per 1000 g. H_2O. This is theoretically better than moles per liter, and leads to quite different values on account of the relatively low water content of the material inside of the cells.

Since in each phase there is electrical neutrality with respect to positive and negative ions, two additional equations must be valid:

$$(B^+)_c = (A^-)_c + (Hb^-)_c, \tag{3}$$

and

$$(B^+)_s = (A^-)_s. \tag{4}$$

Here $(Hb^-)_c$ refers to the concentration of ionized hemoglobin, either oxidized or reduced, in equivalents, not moles, per 1000 g. H_2O. In equation (4) the corresponding concentration of serum proteins is neglected, again as a first approximation. Hydrogen and hydroxyl ions do not appear in either equation because of their relatively low concentrations.

By subtracting equation (3) from equation (2), and adding their difference to equation (4), it is possible to eliminate $(B^+)_c$ and $(B^+)_s$, giving

$$2(A^-)_c = 2(A^-)_s - (Hb^-)_c. \tag{5}$$

By solving this equation for the ratio $(A^-)_c/(A^-)_s$, which is, by equation (1), Van Slyke's r, one obtains

$$r = \frac{(A^-)_c}{(A^-)_s} = 1 - \frac{(Hb^-)_c}{2(A^-)_s}. \tag{6}$$

Equation (6) represents the calculated value of the Donnan ratio for univalent diffusible ions in terms of two variables. It predicts that the formation of more ionized hemoglobin should be accompanied by a more unequal distribution of chloride and bicarbonate ions, as the value of r would then be decreased to a fraction still farther below 1. The equation explains qualitatively the effect of alterations in the oxygen or carbon dioxide content of the blood on the distribution of diffusible ions between cells and serum. According to the diagram of interlocking

chemical equilibria given in the preceding section, an increase in the carbon dioxide content of the blood tends to reduce the concentration of ionized hemoglobin. According to equation (6) such a change should bring the value of r closer to 1. Increased carbon dioxide tension should therefore be accompanied by the passage of chloride ions from the serum to the cells, while the reverse change should be produced by the removal of carbon

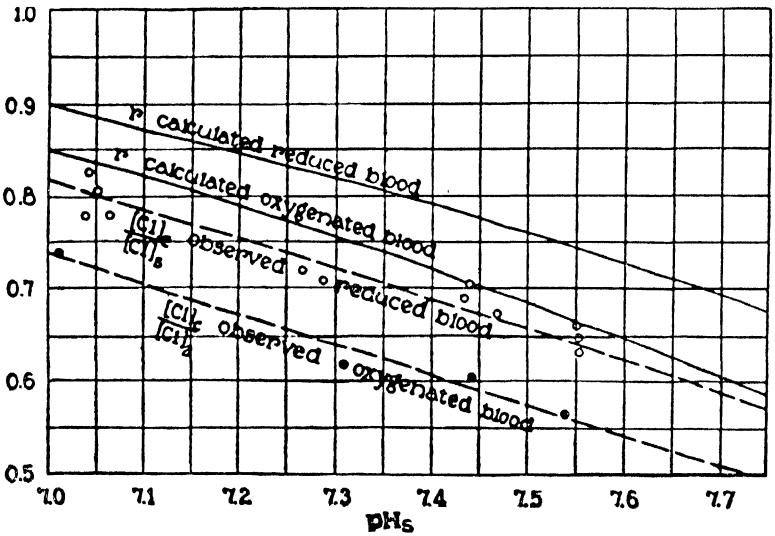

FIG. 19. Effect of changes in pH and in oxygenation on chloride distribution in horse blood. Points and dashed lines are observed values. Full lines are curves of calculated values. (Van Slyke)*

dioxide from the blood, or by an increase in the partial pressure of oxygen in equilibrium with the blood. The equation predicts similar changes in the ratio of the concentrations of bicarbonate ions in the cells and serum.

An approximate quantitative correspondence of theory with experiment was found by Van Slyke and his co-workers in studying the variation of the chloride and bicarbonate ratios with

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changes in the carbon dioxide content of blood *in vitro*. Here the calculated values were not obtained from equation (6), but from a more complicated equation deduced in a similar way, taking into account the osmotic pressure of the hemoglobin, as well as the part played by the ionized serum proteins in the equation of electrical neutrality. The results of these calcula-

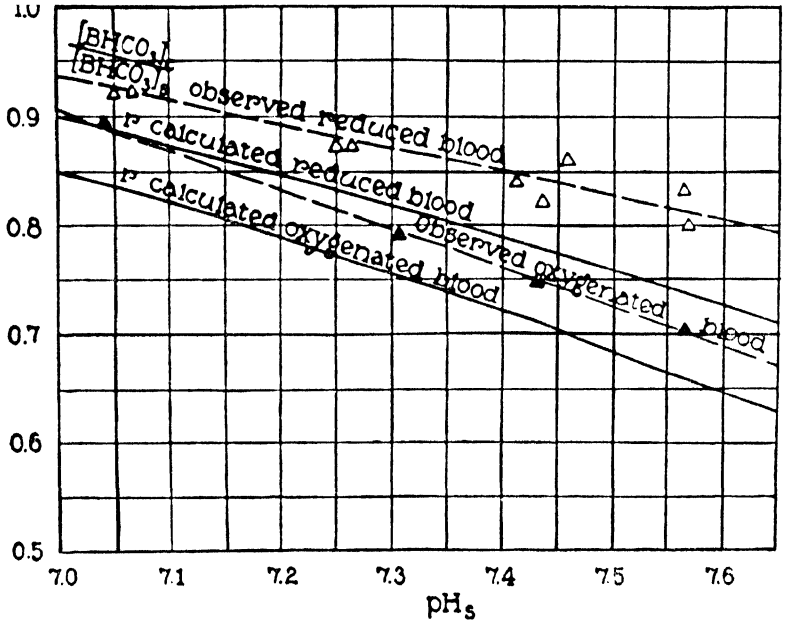

FIG. 20. Effect of changes in pH and in oxygenation on bicarbonate distribution in horse blood. Points and dashed lines are observed values, full lines are curves of calculated values. (Van Slyke)*

tions of Van Slyke are given in Figs. 19 and 20. Here the abscissas are pH values of the serum. These values, of course, become low when carbon dioxide is added to the blood, and high when it is removed. The ordinates are values of the ion ratio r, observed and calculated. It is to be noted that the agreement between theory and experiment was not perfect, the experimen-

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tal r for chlorides being about 0.8 to 0.9 times that for bicarbonates, while the calculated values fell between the two experimental curves. The deviations may be due to the fact that total chlorides were measured by analysis, and not the activity or concentration of chloride ions. Van Slyke's calculations represent at present the best quantitative explanation of the distribution of ions, water, and gases in the blood.

PROBLEMS

1. Make an approximate calculation of the total surface area of the red blood cells in the body of a man weighing 60 kilograms, using any simplifying assumptions which may seem desirable.

2. If the pH of blood serum is 7.4, calculate the ratio of the concentrations of $H_2PO_4^-$ and $HPO_4^=$ in it. The apparent value of the second dissociation constant of phosphoric acid, under the conditions existing in blood, is 2.3×10^{-7}.

3. Which would be the better buffer under the conditions in blood, the phosphate system or the bicarbonate system, if the concentrations of total phosphate and total bicarbonate (ionized +un-ionized) were equal?

4. If the pH of a certain blood serum is 7.42, while that of the cell contents is 7.30, what should be the ratio of the concentrations of $HPO_4^=$ in the cells and the serum? Is this ratio the same as Van Slyke's r? What would be the corresponding ratio for $H_2PO_4^-$? For the total phosphate ion concentrations?

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CHAPTER X

Reaction Velocity and Enzyme Action

Chemical Kinetics. The simplest statement of the law of mass action (Chapter IV) is that the velocity of a chemical reaction is proportional to the product of the active masses (that is, the concentrations) of the reacting substances. For a unimolecular reaction, in which only one type of molecule is reacting, this means that the rate of the reaction at any instant is proportional to the concentration of substance remaining unchanged at that time. This unimolecular law may be expressed by the equation

$$\frac{dx}{dt} = k(a - x) \qquad (1)$$

in which k is a constant of proportionality, a is the initial concentration, x is the change in concentration which has taken place in the time t, and dx/dt is the velocity of the reaction, or the rate of increase, with respect to time, of the concentration of the product (or products) of the reaction. This rate decreases continually as the reaction progresses because the value of $a - x$, the concentration of the reacting substance, is continually decreasing. The constant of proportionality, k, is known as the unimolecular velocity constant or coefficient.

If the progress of the reaction is followed from time to time by some suitable chemical or physical method, a series of corresponding values is obtained for x (or $a - x$) and t. If x is plotted against t for any reaction, whether it follows the unimolecular law or not, a smooth curve is obtained which rises diagonally from the origin and bends gradually, becoming asymptotic to a line parallel to the time axis (Fig. 21, A). The slope of this curve at any point is the reaction velocity, or the change in x per unit of time at the instant of time corresponding to the point in question. The values of this rate, dx/dt, may be obtained by drawing tangents to the curve at various points. To test whether a reaction is unimolecular, one may divide the value of each

rate so obtained by the corresponding experimental value of $a - x$. If the quotients are constant, their value is equal to k, the velocity constant, and the reaction may be said to follow the unimolecular law.

The same test may be made in a much more exact and elegant way by using a form of equation (1) which is obtained by the process known as integration. A student who has had some

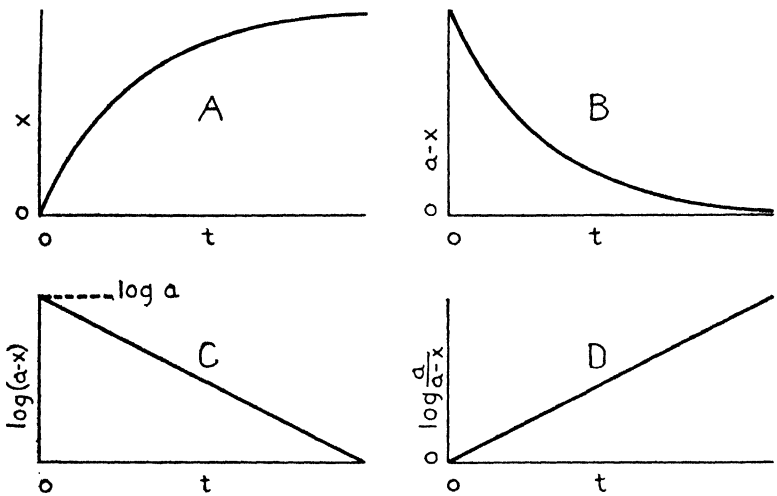

FIG. 21. Reaction velocity curves for a unimolecular reaction.

training in the calculus will readily see that equation (1) may be written

$$\frac{d(a - x)}{a - x} = - kdt$$

or

$$\ln(a - x) = - kt + C.$$

Here ln means logarithm to the base e, and C is a constant of integration. The latter may be evaluated by considering the experimental fact that when $t = 0$, $x = 0$. Hence

$$C = \ln a$$

and equation (1) becomes

$$k = \frac{1}{t} \ln \frac{a}{a - x} \cdot \qquad (2)$$

Equation (2) is the integral form of the unimolecular law. By using the experimental values for a, x, and t, it is possible to calculate a value for k from each observation. Since the ratio between logarithms to the base e and logarithms to the base 10 is a constant factor ($\ln x = 2.303 \log x$), an ordinary table of logarithms may be used. The values of the quantity $(1/t) \log [a/(a-x)]$ are equal to the values of the true velocity constant divided by 2.303, but are equally useful as a test of the constancy of k and hence of the applicability of equation (2) to experimental data.

If $a - x$ is plotted against t, the curve obtained is like that of x against t, but inverted (Fig. 21, B). If the logarithm of $a - x$ is plotted against t, equation (2) predicts that a straight line should be obtained, having a slope equal to $-k/2.303$. When $x = 0$, which is true when $t = 0$, $\log (a - x) = \log a$. Hence the plot of $\log (a - x)$ against t gives a simple method of obtaining a, the initial concentration, by extrapolation. This method of getting a is sometimes useful if the experimental determination of $a - x$ is more reliable than that of a, but it is justified only if the observed points actually fall on a straight line on the semi-logarithmic plot (Fig. 21, C).

Equation (2) also implies that a plot of $\log [a/(a-x)]$ against t should be a straight line passing through the origin, with a positive slope equal to $k/2.303$ (Fig. 21, D).

Another consequence of equation (2) is that the velocity constant is independent of the initial concentration in a unimolecular reaction.

There are also formulas for bimolecular reactions and those of higher order, all based on the law of mass action. Several of these equations have been found to agree with experimental data obtained in the study of chemical reactions. The velocity constant is in each case a measure of the velocity throughout the reaction.

Experimentally the unimolecular equation fits many reactions which appear to involve more than one type of molecule. The classical test of the theory of reaction velocity was made with the hydrolysis of cane sugar in the presence of an acid, the

reaction being

$$\underset{\text{(sucrose)}}{C_{12}H_{22}O_{11}} + H_2O \rightarrow \underset{\text{(glucose)}}{C_6H_{12}O_6} + \underset{\text{(fructose)}}{C_6H_{12}O_6}.$$

This follows the unimolecular formula because in most experiments the decrease in the concentration of water during the reaction is negligible.

Effect of Temperature on Reaction Velocity. Practically all chemical reactions go faster at higher temperatures. The temperature coefficient of a chemical reaction, or the ratio of comparable velocities (or, better, velocity constants) for the same reaction at two different temperatures, is not a constant, but its value depends on the temperature range in question. As a rough approximation it may be said that the temperature coefficients for most chemical reactions over an interval of 10°C., in the vicinity of room temperature, or from 25 to 35°C., are apt to lie between 2 and 4. This approximate generalization has sometimes been used in attempting to decide whether an unknown reaction is of a chemical or a physical nature; most purely physical processes, such as diffusion, have lower temperature coefficients, usually closer to 1 than 2. The symbol Q_{10} is sometimes used for a temperature coefficient over an interval of 10°C. It is somewhat clearer to express such a coefficient as a ratio of velocity constants at two temperatures designated by subscripts, as k_{25}/k_{15}, k_{35}/k_{25}, etc.

For many reactions it has been found that the logarithm of the velocity constant is a straight line function of the reciprocal of the absolute temperature, or

$$\log k = A - \frac{B}{T} \tag{3}$$

where A and B are empirical constants, A being the intercept of the line and $-B$ its slope. Such a straight line may equally well be represented by the equation

$$\ln \frac{k_1}{k_2} = \frac{\mu}{R}\left(\frac{1}{T_2} - \frac{1}{T_1}\right) \tag{4}$$

which is identical in form with an equation deduced by van't Hoff for the relation between the equilibrium constant of a reaction (not the velocity constant) and the temperature. In van't Hoff's equation μ is the heat evolved in the reaction, on the assumption that it is identical at the temperatures T_1 and T_2, and R is the gas constant (roughly 2, more exactly 1.9869 calories). Attempts have been made by Arrhenius and others to invent a mechanism by which equation (4), in terms of velocity constants, can be derived from the van't Hoff equation for equilibrium constants. As a result of these theories the value of μ in equation (4) is often taken to be the number of calories of energy required to activate one mole of the reacting substance so that the reaction will take place, and μ has been called the "critical increment" or the "temperature characteristic" of the reaction.

If the plot of log k against $1/T$ really gives a straight line, then equation (3) or (4) is a useful empirical expression for the effect of temperature. The value of μ in equation (4) may be readily obtained from the slope of the line, $-B$ in equation (3), since $\mu/R = 2.303\ B$ or $\mu = 4.575\ B$ calories. The use of μ in describing temperature effects is preferable to that of Q_{10} because, if the line is really straight, the value of μ must be constant and independent of the temperature, while that of Q_{10} must vary with the temperature.

The effect of temperature on the rates of biological processes, such as heart beats and respiratory movements, has been found to be represented fairly well by equation (4). In certain cases when the logarithm of a rate is plotted against the reciprocal of the absolute temperature, the points appear to fall on two or more straight lines with different slopes, indicating abrupt changes in the value of μ at definite temperatures. Interesting inferences have been made as to the nature of possible chemical processes controlling such biological rates (33).

Catalysis. Any substance which alters the velocity of a chemical reaction without being used up or changed in the process is called a catalyst for that reaction, and such alteration in reaction velocity is called catalytic action or catalysis. Most

catalysts accelerate reactions, but cases of negative catalysis are also known. In homogeneous catalysis the molecules of the reacting substances and the catalyst are uniformly distributed throughout the available space; examples are the hydrolysis of sucrose in the presence of an acid in aqueous solution, and the hydrolysis of an ester dissolved in water in the presence of an alkali. In heterogeneous catalysis the system contains at least two phases, which means that not all of the molecules are uniformly distributed. Examples are the decomposition of hydrogen peroxide in the presence of platinum in colloidal solution, and the union of hydrogen and oxygen gases to form water in the presence of spongy solid platinum. In each case the reaction takes place at the phase boundaries between the platinum and the remainder of the mixture. One characteristic property of catalytic action is the tremendous effect produced by the presence of small traces of the catalytic substance.

Homogeneous catalysis has often been explained by assuming the catalyst and the reacting substances to form an addition compound which then breaks up to give the reaction products and the free catalyst, the velocity of the reaction at any instant being proportional to the concentration of the addition compound. Only a few such compounds have been isolated. Heterogeneous catalysis is usually explained on the basis of adsorption, the reacting substances being supposed to be more active chemically when concentrated on the surface of the catalyst. There is no generally accepted theory which explains all types of catalysis.

Kinetics of Catalyzed Reactions. In general it is found that the velocity of a catalytic reaction is proportional to the amount or concentration of the catalyst. In the classical example of sucrose hydrolysis the rate varies as the concentration of hydrogen ions. In such cases the experimental velocity constant is proportional to the concentration of the catalyst, but in each experiment the course of the reaction follows one of the theoretical velocity equations. In the case of some heterogeneously catalyzed gas reactions, the velocity is proportional to a fractional exponent of the gas pressure. This would be expected if the

velocity were controlled by the amount of gas adsorbed and if the adsorption of gas followed Freundlich's equation (Chapter VII, equation (1)).

Enzymes. Enzymes are organic catalysts occurring in living cells or secreted by them. Most reactions in living organisms seem to be catalyzed by enzymes. Although Pasteur[1] did important pioneer work in pointing out the influence of microorganisms in fermentation processes or enzyme reactions, he seems to have been wrong in believing that such processes could not take place in the absence of living cells. Buchner (22) showed in 1897 that alcoholic fermentation could be catalyzed by a cell-free yeast extract prepared by grinding the cells with sand. Since that time it has generally been believed that each enzyme is a definite chemical substance, but none has yet been so fully identified that its chemical constitution is known. In recent years considerable progress has been made, for Sumner (116) has succeeded in crystallizing urease from jack beans, Northrop (92, 96) has reported the isolation of crystalline pepsin and trypsin from materials of animal origin, and Sherman (24) has obtained crystalline amylase of pancreatic origin. All of these crystalline preparations are of protein nature. It should be added that the evidence so far published has not convinced all workers that the crystals and the enzyme are, in any case, one and the same substance.

Specificity is another characteristic of enzyme action. Pepsin and trypsin catalyze the hydrolysis of proteins but not of carbohydrates; invertase (also called sucrase or saccharase) hydrolyzes sucrose and raffinose but not starch or maltose; maltase hydrolyzes maltose and alpha glucosides but not beta glucosides while the reverse is true of emulsin. This specificity, however, differs only in degree from that shown by inorganic catalysts; for example, it has been found (103) that the decomposition of

[1] The name of Louis Pasteur (1822–1895), founder of the science of bacteriology and one of the greatest figures in modern science, is of course familiar to all. He held professorships of chemistry in several French universities, finally at the Sorbonne in Paris. His chief chemical work dealt with the optical isomerism of organic compounds (separation of *d*- and *l*-tartaric acids) and with fermentation.

formic acid to produce carbon dioxide and hydrogen is catalyzed by metals such as copper or nickel, while an oxide catalyst such as thoria causes the same substance to be decomposed quantitatively into carbon monoxide and water. Other examples of specificity of inorganic reagents are found in the separations of analytical chemistry; it has been stated (41) that the specificity of enzymes is no more mysterious than the specificity of hydrogen sulphide in the separation of some metallic ions from others.

Reversibility of Enzyme Action. If an enzyme is a true catalyst in the sense that it is not changed by the reaction which it accelerates, its presence should not affect the value of the equilibrium constant of a reversible reaction. Since an equilibrium constant is the ratio of the velocity constants of two opposing reactions, it follows that an enzyme which accelerates a reversible reaction in one direction should have an identical effect on the velocity of the reverse reaction. This means that an enzyme which is known to catalyze a hydrolysis, for example, should also catalyze a synthesis of the initial substance by dehydration of the products of the hydrolytic reaction. This theoretical prediction was made by van't Hoff (123) in 1898, shortly after Croft Hill (62) had shown experimentally that a yeast extract, which was known to hydrolyze maltose to form glucose, could also effect the synthesis of a di-saccharide from glucose. A number of other cases of enzymic synthesis have been reported, particularly with the lipases or fat-splitting enzymes. Further knowledge of such catalytic synthesis should be of fundamental importance in physiology in connection with the formation of proteins, carbohydrates, and fats in living organisms. Synthesis of protein by pepsin has been reported (19), but the results appear not to be universally accepted as conclusive. In the case of invertase, the enzyme which has been most widely and carefully studied, no valid evidence of synthetic action has been obtained.

Theories of Enzyme Action. Just as there is no general theory of catalysis, so there is none for enzyme action. Since enzymes during their activity are in the colloidal state, or at least have so far been inseparably associated with material in that state, the same sort of disagreement exists as to possible explanations

of their behavior which has been mentioned in connection with proteins and other hydrophilic colloids. Bayliss[2] (11, 12) emphasized the heterogeneous character of colloidal enzyme solutions, and postulated adsorption as an essential step in all enzyme reactions. A number of other workers have preferred to treat enzymes as molecules in true solution, applying the law of mass action to an assumed reversible combination of enzyme and substrate (21, 60). (The term substrate is applied to the principal reacting substance, other than water, in a reaction catalyzed by an enzyme.) The most widely accepted of these intermediate compound theories is that of Michaelis (86), which assumes a reversible combination of the enzyme not only with the substrate but also with the products of the reaction. This theory has been adopted by such well known investigators as Euler[3] (40) and Willstätter[4] (129). It has been shown by Northrop[5] (91), however, that the hydrolysis of proteins by pepsin or trypsin may be explained by the use of the mass law, but without the assumption of compound formation between enzyme and substrate. He found that his experiments could best be ex-

[2] William Maddock Bayliss (1860–1924) was Professor of General Physiology in University College, London. He was widely known for his research in the field of enzyme action and various other branches of physiology, and particularly for his book, *Principles of General Physiology*. In this book he emphasized the need for an understanding of physical chemistry, and particularly of colloid chemistry, for the solution of fundamental physiological problems. His smaller book on enzymes (*The Nature of Enzyme Action*) was for many years the best summary of the subject in English. It has now been supplemented by Haldane's monograph, which is cited at the end of this chapter.

[3] Hans von Euler-Chelpin (1873–), Professor of Chemistry in the University of Stockholm, is one of the most productive workers in the field of enzyme chemistry. His book (40) may be recommended for reference.

[4] Richard Martin Willstätter (1872–), Professor of Chemistry in the University of Munich, was known as one of the foremost organic chemists long before he turned his attention to enzymes. His work in the latter field has included quantitative studies of methods of preparing enzymes in highly active form.

[5] John Howard Northrop (1891–) is a Member of the Rockefeller Institute for Medical Research, Princeton, New Jersey. He has made extensive use of the principles and methods of physical chemistry in studying the behavior of enzymes.

plained by assuming that the products of the reaction, or added inhibiting substances, entered into reversible combinations with the enzyme, only the free enzyme being active. It was also necessary to take into account the spontaneous inactivation of these enzymes during the course of the reaction.

Without entering into further details of such theories, a brief summary will be given of some of the facts on which they are based.

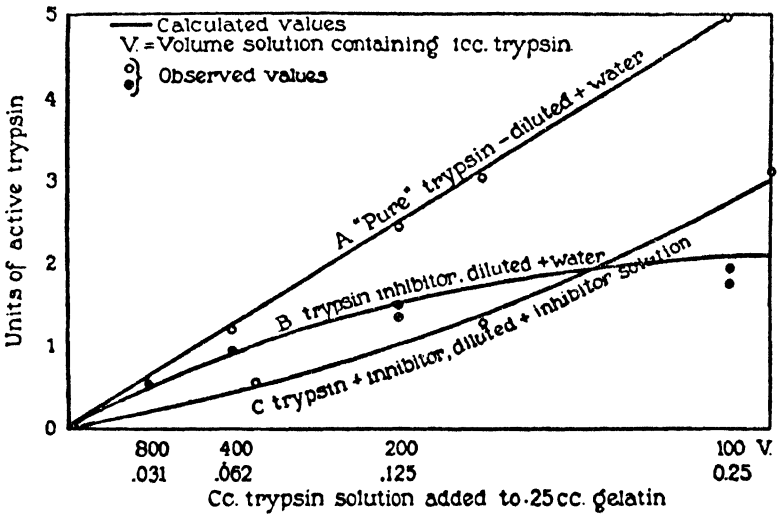

FIG. 22. Effect of enzyme concentration on reaction velocity; direct proportionality was obtained only with purified enzyme. Experiments with gelatin and trypsin. (Northrop)*

Effect of Enzyme Concentration. In the case of invertase, it has been shown by Michaelis (86), Nelson[6] (90), and others that the reaction rate is directly proportional to the concentration of enzyme. For proteolytic (protein-splitting) enzymes it was long believed that this was not true, as the rate appeared to increase less rapidly than the enzyme concentration. Northrop

* J. H. Northrop, *J. Gen. Physiol.*, 4, 256 (1921–22).

[6] John Maurice Nelson (1876–) is Professor of Organic Chemistry in Columbia University, New York City. His researches on invertase action represent probably the most carefully controlled and painstaking experiments which have been done with any enzyme. His papers have appeared in *J. Am. Chem. Soc.* and *J. Biol. Chem.*, from 1914 to the present.

(91) was able to show that for pepsin and trypsin this peculiar behavior was due to the presence of inhibiting impurities in the ordinary enzyme solutions. The more dilute the enzyme solution, the less was the effect of the impurities, presumably owing to a reversible dissociation of the combination between them and the enzyme. By using solutions freed from such impurities, he obtained a direct proportionality between reaction velocity and enzyme concentration, as illustrated in Fig. 22, Curve A. Curve B shows the result obtained with ordinary impure en-

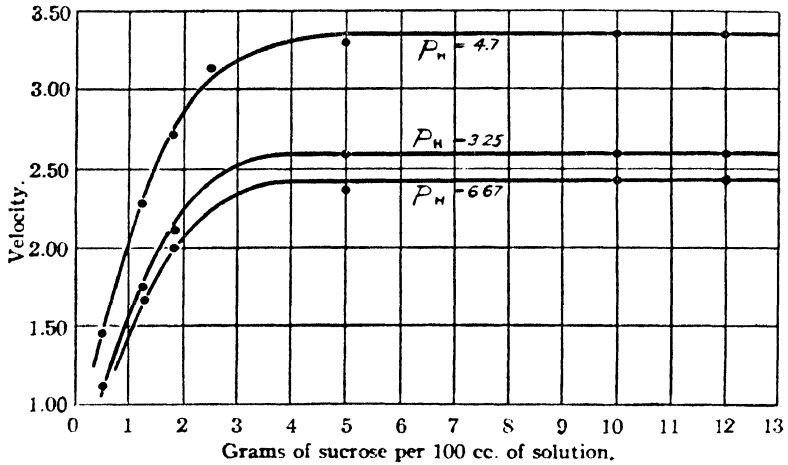

FIG. 23. Effect of substrate concentration on reaction velocity, illustrating the limiting or saturation value at higher concentrations. Experiments with sucrose and invertase. (Nelson and Bloomfield)*

zyme solutions, while the opposite curvature of C was obtained by diluting the enzyme with an inhibiting solution, the activity decreasing more rapidly than the concentration. Northrop explained this result as due to repression of the dissociation of the enzyme-inhibitor compound, in accordance with the law of mass action as applied to equilibrium in a reversible reaction.

Effect of Substrate Concentration. The rate of enzyme reactions in general increases less rapidly than the initial concentration of substrate, eventually, in many cases, reaching a limiting or saturation value as the substrate concentration is in-

* J. M. Nelson and G. Bloomfield, *J. Am. Chem. Soc.*, **46**, 1027 (1924).

creased (Fig. 23). Bayliss interpreted this as due to a saturation of the adsorbing surface of the enzyme particles. Michaelis explained it, somewhat more quantitatively, by assuming reversible compound formation between the enzyme and the substrate, the amount of the compound present being governed by the law of mass action. Northrop has reported experiments which seem to show that the surface saturation theory is not applicable to pepsin and trypsin, and that the intermediate compound theory cannot be valid for trypsin. He explained the kinetics of trypsin digestion, including the effect of substrate concentration, by assuming reversible combination between the enzyme and the products of the reaction, as was mentioned above. Nelson (89) has found that the rate of invertase action is greatly decreased, in experiments with very high substrate concentrations, to a value far below the maximum or saturation value, and he believes that the reduction of the concentration of water by the presence of large amounts of sugar is an important factor in explaining such effects.

Effect of Hydrogen Ion Concentration. In the classical paper in which hydrogen ion measurements were first applied in biological chemistry, Sørensen (110) showed that the rates of the reactions catalyzed by invertase, catalase, and pepsin were markedly influenced by the acidity of the solutions in which the reactions took place. Other conditions being constant, each enzyme had an optimum range of pH values, the velocity of the reaction being less when the acidity was too high or too low. This has been found to be quite general for all enzymes.

Michaelis (84) pointed out that the curve of reaction velocity against pH is quite similar to the dissociation residue[7] curve of an amphoteric electrolyte having its isoelectric point at the pH of optimum enzyme activity. In the case of invertase the resemblance is particularly marked on the alkaline side of the optimum pH, the activity curve from about pH 5 to 8 being almost identical with the theoretical dissociation residue curve of

[7] Michaelis defined the dissociation residue of an ampholyte as the un-ionized fraction. In terms of the *Zwitterion* theory, it would mean the fraction in either the *Zwitterion* or un-ionized form.

a univalent weak acid (like Fig. 8, p. 67, but inverted). Michaelis accordingly proposed the theory that this enzyme actually was an amphoteric electrolyte, the catalytically active part being the undissociated molecule. He assumed the decrease in activity with increased pH to be due to increased ionization of an acidic group, whose ionization constant he obtained graphically from the pH corresponding to half of the maximum activity of the enzyme. Similar relations were found with other enzymes, but in some cases the active catalyst appeared to be an ionic form of the enzyme.

Northrop (91) has obtained data, using proteolytic enzymes, showing that changes in enzyme activity with pH are related to the state of ionization of the protein rather than to that of the enzyme. He found a considerable degree of parallelism between the activity-pH curves for the enzymes pepsin and trypsin and the titration or combination curves of several proteins used as substrates. He also showed (91), by studies of the distribution of pepsin and trypsin between particles of undissolved gelatin and the surrounding solution, that these enzymes were distributed in the same way as simple univalent diffusible ions, chloride ion and hydrogen ion. By this application of the Donnan theory of membrane equilibria he concluded that trypsin behaved like a univalent positive ion, and pepsin like a univalent negative ion.

Kinetics of Enzyme Reactions. In only a few cases, under experimental conditions most carefully devised with a view to the elimination of complicating effects, has it been found that any enzyme reaction followed the unimolecular law or any other of the theoretical equations of chemical kinetics. In most cases the experimental course of the reaction has proved to be more complicated than any simple equation would predict. In the case of invertase action, by assuming reversible compound formation between the enzyme and the substrate and between the enzyme and the reaction products, Michaelis (86) found it possible to formulate a kinetic expression for the course of the reaction which fits the facts fairly well, at least for the first half of the reaction. In order to get a more exact expression for the whole

course of the reaction, it was found necessary to use an empirical equation containing three arbitrary constants (88), which has proved useful in studying the normal course of the reaction. In studying the action of pepsin and trypsin, Northrop (91) has found it preferable to study varied sets of experimental conditions separately, rather than to attempt to formulate a single kinetic equation which would fit the course of the reaction under all conditions. In this way he has found conditions such that the results are fitted by the simple unimolecular law as applied either to the velocity of the hydrolysis of proteins or to the velocity of the spontaneous destruction of the enzyme. The kinetics of enzyme reactions is a problem which, although it has fascinated many workers, is still far from completely solved.

PROBLEMS

1. A certain enzyme reaction was found to be 35.4 per cent complete after 60 minutes. If the unimolecular law applies, calculate the extent of the reaction after 300 minutes.

2. The reaction of Problem 1 was actually 94.5 per cent complete after 300 minutes. Did the unimolecular law apply? If not, did the value of the velocity coefficient increase or decrease as the reaction went on?

3. What is the relation between the velocity constant of the unimolecular formula and the time required for half of the reacting substance to be transformed?

4. If a reaction follows the unimolecular law, what fraction of the reacting substance will have been transformed after the reaction has proceeded for a time equal to the reciprocal of the velocity constant? This problem should be answered both with reference to the true velocity constant, using logarithms to the base e, and the relative velocity constant, using logarithms to the base 10.

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CHAPTER XI

Oxidation-Reduction Potentials; Phase Boundary Potentials; Electrokinetic Phenomena

This chapter deals with certain types of electrical potential differences which are not necessarily related to one another; the only reason for considering them together at this point is that they have all been used in attempts to interpret physiological facts.

Electronic Idea of Oxidation. Oxidation is now usually defined as the loss of electrons by an atom or ion, and reduction as the reverse. The electrical nature of these chemical processes may be demonstrated by simple experiments devised by Ostwald, illustrating what he called chemical action at a distance (115). A solution of ferrous chloride, free from ferric ions, is put into a beaker, and a solution of ferric chloride, free from ferrous ions, into another beaker. Electrodes of platinum or some other inert metal are dipped into the two solutions and connected to an electric battery, the electrode in the ferrous chloride solution being joined to the positive pole. The circuit is completed by connecting the two solutions by a salt bridge, which may be an inverted U-tube containing a solution of potassium chloride in agar jelly (Fig. 24). After the current has been allowed to flow for a few minutes, it is possible to prove by qualitative tests that ferric ion is now present in the beaker which contained the ferrous salt, and that ferrous ion has been formed in the solution of the ferric salt. Quantitative analysis shows that the amounts of these ions formed by the same current are chemically equivalent. (The current must, of course, not be allowed to flow long enough for the migration of these ions from one beaker to the other through the salt bridge.) If the polarity of the electrodes is reversed by changing the connections to the battery, current will flow in the opposite direction and the chemical reaction will be reversed. The experiment shows that oxidation of ferrous ion and reduction of ferric ion can be induced by the action of the electric current, without actual contact of the reacting ion

species. The only contact is with the non-reactive ions of the salt bridge and the chemically inert electrodes. The chemical reaction is therefore ascribed to the transfer of electrons, and it may be written

$$Fe^{++} \rightleftharpoons Fe^{+++} + e.$$

In this electrochemical equation the symbol e represents 1 faraday of electrons, or 96,500 coulombs of negative electricity. The

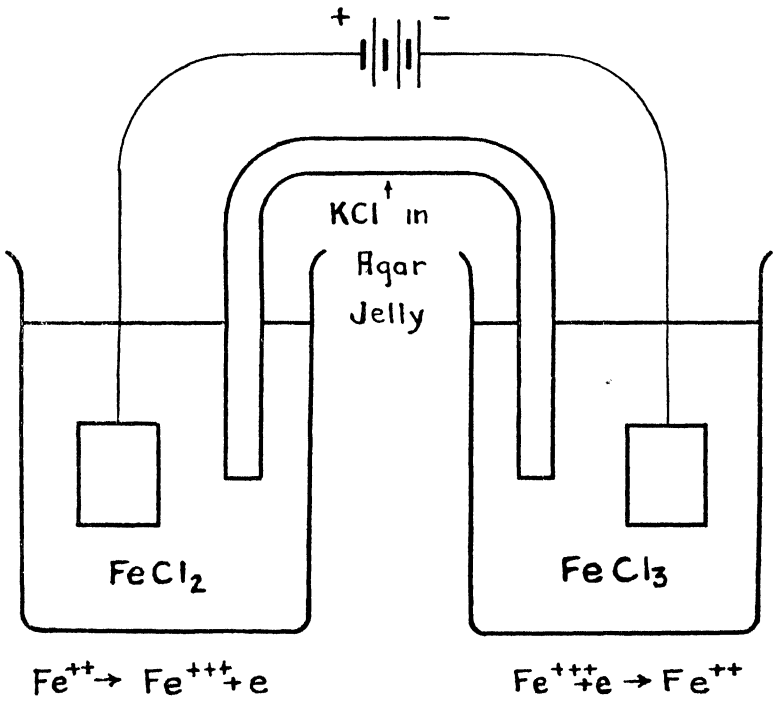

FIG. 24. Oxidation and reduction by an electric current.

equation states that the reaction is reversible and stoïchiometric with respect to electricity as well as matter.

The converse experiment may be performed in a similar way. If solutions of an oxidant and a reductant are put in separate beakers provided with electrodes, and the latter are connected, not to a battery, but to a galvanometer or other instrument for detecting the passage of a current, it is often found that a cur-

rent flows through the wire connecting the electrodes when the circuit is completed by a salt bridge. In other words, a galvanic cell is formed in which the cell reaction is the transfer of electrons from one ion to the other. The electrode in the solution of the reductant becomes negative, since oxidation sets free electrons. The fact that a current flows between the electrodes in this experiment indicates that there is a potential difference between each electrode and the solution bathing it, and that these potential differences are not the same.

Electrode Potentials of Oxidation-Reduction Systems. It is possible to make a definite measurement of the potential difference between such an electrode and the solution only if the solution contains finite amounts of both the oxidized and the reduced forms of the same substance There is a quantitative relation between such potential differences and the proportions of the oxidized and reduced forms, which was first studied experimentally for the case of ferrous and ferric ions by Peters (76), a student of Ostwald. He prepared mixtures of ferrous and ferric chlorides in known but varied concentrations, adding hydrochloric acid to repress hydrolysis, and measured the E.M.F. between a platinum electrode dipped into these mixtures and a 1.0 *N* KCl calomel half cell, the latter being connected to the solutions by a salt bridge. His cells were therefore

$$\text{Hg, HgCl in KCl(1.0 } N\text{), KCl, } FeCl_2 + FeCl_3 \text{ in HCl, Pt(+)}$$

and the experimental arrangement was similar to that shown in the right hand portion of Fig. 10 (page 86) (omitting the tube for saturating the hydrogen and the inlet and outlet tubes for this gas). The electrode used for such measurements is generally a piece of bright platinum or gold foil, rather than a platinized wire or foil. Some of the results obtained in this pioneer work of Peters are given in the accompanying table. The constancy of the figures in the last column shows that the observed E.M.F. was a linear function of the logarithm of the ratio of the concentrations of ferric and ferrous ions, the slope of the line being 0.0575, which is the value of 0.0001984 T at the temperature of

$R=\frac{(Fe^{+++})}{(Fe^{++})}$	log R	0.0575 log R	E, volts	E-0.0575 log R
0.111	−0.955	−0.055	0.375	0.430
0.429	−0.367	−0.021	0.408	0.429
1.000	0	0	0.427	0.427
2.33	0.367	0.021	0.448	0.427
9.00	0.955	0.055	0.483	0.428

the experiments, 17°C. In other words, the table shows that, for these experiments,

$$E = \text{Constant} + 0.0001984\ T \log R.$$

Peters found the platinum electrode to be positive with respect to the calomel half cell. Since the 1.0 N KCl calomel half cell is about 0.285 volt more positive than the molal hydrogen electrode at 17°C., his readings may be reduced to the usual arbitrary standard[1] by adding 0.285 volt to each E.M.F. value. Peters' equation for oxidation-reduction potentials, in terms of the molal hydrogen electrode as zero, may then be generalized to read

$$E_h = E_0 + \frac{0.0001984\ T}{N} \log \frac{(\text{oxidant})}{(\text{reductant})}. \qquad (1)$$

Here E_h is the observed potential difference, with respect to the molal hydrogen electrode, E_0 is a constant for each system if the temperature is constant, N is the number of faradays of electrons transferred per mole in the electrochemical equation, and (oxidant) and (reductant) are the activities of the oxidized and reduced forms, when these differ only in their electron content. The value of the constant E_0 is characteristic of the particular oxidation-reduction system under investigation, being the value of E_h when the activities of the oxidized and reduced forms are

[1] It is customary to define a scale for electrode potential differences, or the potential difference between each electrode and the solution in contact with it, by assuming that of the molal hydrogen electrode (defined in Chapter VI) to be zero at all temperatures. Two opposite conventions are in use as to the sign of single electrode potentials. Confusion may be avoided if an electrode in the expression for a cell or half cell is always labelled with a plus or minus sign.

equal; E_0 is often called the normal or molal electrode potential for the system.

While equation (1) was first verified in the case of a system composed of an inorganic oxidant and reductant, it has recently been found to apply equally well to many organic systems, such as mixtures of quinone and hydroquinone, their substitution products, various indigo derivatives, and indophenols. In each case the value of E_0 for a system is a quantitative measure of its tendency to react with other systems. A system for which E_0 has a positive value (that is, the electrode in the mixture of equal parts of oxidant and reductant is positive with respect to the molal hydrogen electrode) will oxidize, and be reduced by, systems for which E_0 is less positive or more negative. A table of molal electrode potentials for oxidation-reduction reactions may be used to predict chemical reactions, just as the familiar table of the molal electrode potentials (electromotive series) of the metals is used. In fact, the two tables really represent the same thing, for the formation of positive ions from a metallic element is oxidation just as much as the formation of Fe^{+++} from Fe^{++}. Modern text books usually present molal electrode potentials in a single table, irrespective of whether the electrode material takes part in the reaction or not.[2]

The equation for single electrode potentials has been given in the preceding discussion simply as an empirical equation which agrees with certain experimental data. For the case of a reversible cell without liquid junction, in which one of the electrode reactions is the oxidation-reduction reaction to be studied, it is possible to derive equation (1) by means of thermodynamics. By the methods outlined by Lewis and Randall (75), involving measurements of various types of cells without liquid junction and extrapolation to unit activity to get the proper E_0 values, it is possible to get a rigidly accurate E_0 value for the single electrode potential of an oxidation-reduction system. So far, however, most of the measurements of these potentials have involved the use of a salt bridge and are therefore accompanied

[2] For tables, see Cartledge, p. 426; Gillespie, p. 150; Hammett, p. 124; Lewis and Randall, p. 433; or Noyes and Sherrill, p. 260.

by some uncertainty (of the order of a few millivolts) as to liquid junction potentials.

Organic Systems; Influence of Hydrogen Ions. In the case of a simple inorganic oxidation such as that of ferrous ion to ferric ion, the E_h and E_0 values should theoretically be independent of the hydrogen ion activity of the solution. In other cases the hydrogen (or hydroxyl) ion may appear in the electrochemical equation, and hence the values obtained for E_h may depend markedly on the pH. This is particularly true in the case of organic systems, for here the reductant is usually the negative ion of a weak acid. For example, consider the oxidation of hydroquinone to form quinone,

$$C_6H_4(OH)_2 \rightleftharpoons C_6H_4O_2 + 2H^+ + 2e.$$

On comparing this with the electrochemical equation for the oxidation of ferrous ion,

$$Fe^{++} \rightleftharpoons Fe^{+++} + e,$$

it is seen that the oxidant in the hydroquinone system is not quinone alone, but $C_6H_4O_2 + 2H^+$, and that the value of N is 2. Since the ratio whose logarithm appears in equation (1) is a mass action expression, the equation for this case becomes

$$E_h = E_0 + \frac{0.0001984\ T}{2} \log \frac{(C_6H_4O_2)(H^+)^2}{(C_6H_4(OH)_2)}$$

or

$$E_h = E_0 + \frac{0.0001984\ T}{2} \log \frac{(C_6H_4O_2)}{(C_6H_4(OH)_2)} + 0.0001984\ T \log (H^+). \qquad (2)$$

Equation (2) is an approximation which holds only under certain conditions, but it has been extremely useful. It may be derived from free energy considerations, using the law of mass action for the ionization of hydroquinone as a dibasic acid, but it appears in this form only if it is justifiable to replace the activities of the organic compounds by concentrations and if the acidity is great enough (pH less than about 8) so that certain terms may be neglected.

Equation (2) contains two variables other than the temperature, the ratio of the total concentrations of quinone and hydroquinone, and the hydrogen ion activity. The latter may be held constant by the use of buffers, or by sufficient concentrations

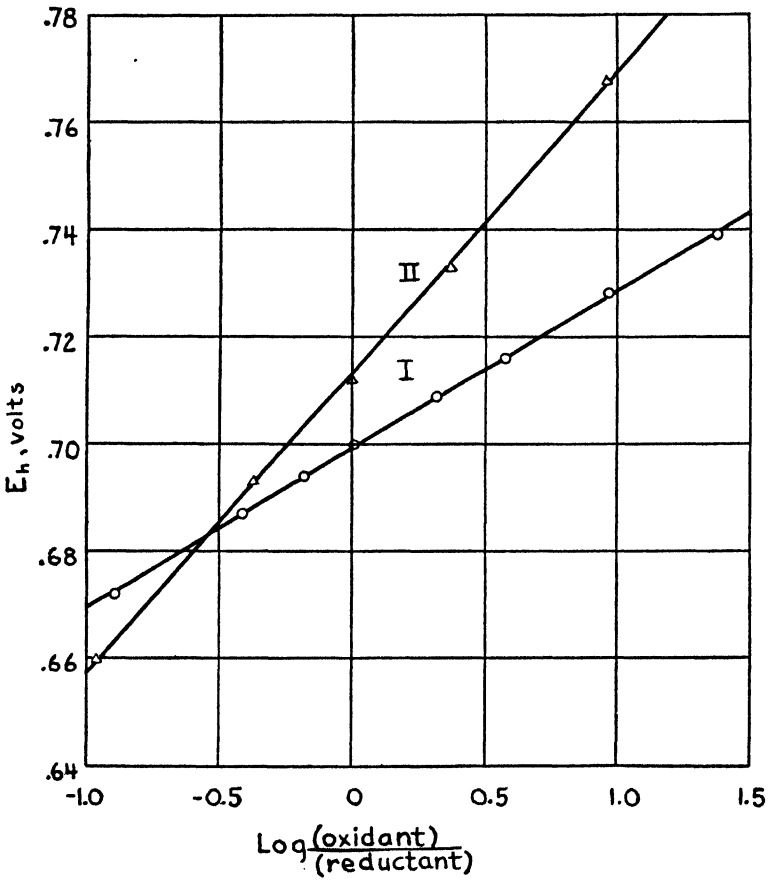

FIG. 25. Experimental verification of equations for oxidation-reduction potentials. I, quinone: hydroquinone. II, $Fe^{+++}:Fe^{++}$.

of a strong acid. Then the E.M.F. observed ought to vary as a linear function of the logarithm of the ratio of the concentrations of quinone and hydroquinone. That this is actually true was proved independently by several workers. The ratio was varied either by making up solutions to contain known amounts of the

oxidant and the reductant, or by titrating the reductant with an oxidizing solution of higher potential, the pH being held approximately constant in either case. The agreement with the equation of certain data (72) obtained by the latter method at 25°C. is illustrated in Curve I of Fig. 25. The experiment consisted in titrating hydroquinone in aqueous hydrochloric acid with potassium dichromate made up in hydrochloric acid of the same concentration. The oxidation potential of the dichromate system is enough higher than that of the hydroquinone system so that all of the dichromate reacts quantitatively as long as there is an excess of hydroquinone; the fraction of the latter which has been oxidized can therefore be obtained by simple proportion from the volume of the dichromate solution added and the known concentrations of the reactants. The line of Curve I, which was drawn through most of the points plotted, has a slope equal to 0.0295, while equation (2) predicts that the slope should be 0.05915/2 or 0.0296. The agreement is better than might be expected from such a graphical method.

Fig. 25 also shows, in Curve II, a similar test of equation (1), using the original data of Peters obtained at 17°C. The points fall fairly well on a straight line of slope 0.0562, which is reasonable agreement with the theory, which predicts 0.0575 for the slope.[3]

Fig. 26 represents the same data as Fig. 25, with E_h plotted against the percentage of the total reactive material in the oxidized form. The curves are quite similar to the dissociation curve of a weak electrolyte (Fig. 8), but differ from this and from one another in the steepness of the slope at the mid point, corresponding to the different coefficients of the logarithmic terms in equation (2) of Chapter V and equations (1) and (2) of this chapter. These curves are typical of those that have been obtained with many reversible systems, both organic and inorganic. The curves for different systems may differ in slope

[3] These data of Peters do not give exactly the correct E_0 for the iron system, as he found the value to increase with dilution. An extrapolation of some of his values to infinite dilution gives 0.744 for 17°, while Lewis and Randall, by an entirely different method, calculated $E_0 = 0.7467$ for 25°.

depending on the value of N in equation (1) or (2), and in their position along the scale of ordinates, corresponding to different

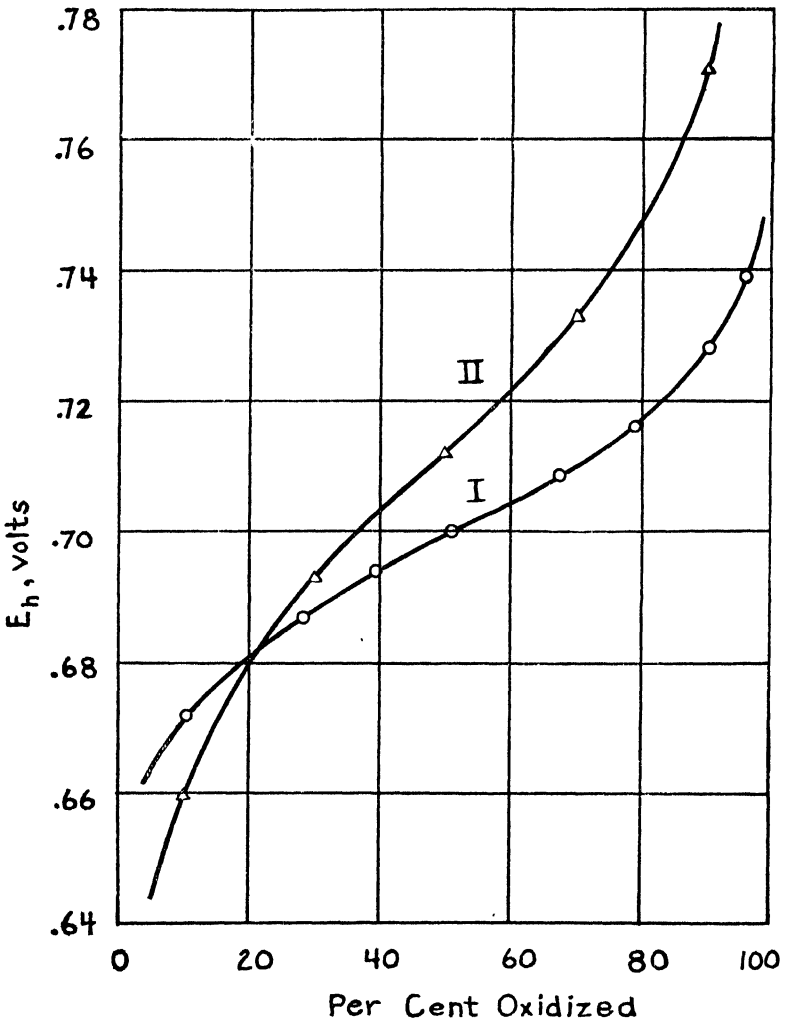

FIG. 26. Variation of oxidation-reduction potentials with per cent oxidized. I, quinone : hydroquinone. II, $Fe^{+++} : Fe^{++}$.

values of E_0, but the general trend of the curves is similar for all reversible systems. If two curves for different systems intersect, provided that they have been obtained under comparable

conditions, the value of the abscissa corresponding to the point of intersection gives the composition of the solutions such that no reaction will take place if solutions containing the two systems are mixed. If a solution containing one system at a higher E_h value is added to one of another system at a lower E_h value, the latter will be partly oxidized by the former, the resulting E_h value of the mixture being intermediate between the two original values for the separate solutions.

In the case of many organic systems, one component is highly colored. The changes in color produced by adding small amounts of such a component to a system under investigation have been used by W. M. Clark (27) and others as a means for the approximate determination of oxidation potentials without electrometric measurement. While such colorimetric indicators of oxidation and reduction have not yet yielded data of precision comparable to that in pH determinations by indicators, the method is convenient for many biological experiments. It is of interest that the first biological application of such indicators was made by Ehrlich.[4]

The oxidation potentials of a number of organic systems, as indicated by equation (2), vary with the pH of the solution in which they are measured. In such cases a curve of the shape of Curve I, Fig. 26, is obtained only if the measurements are carried out at constant pH. From equation (2) it may be seen that E_0 may be obtained experimentally as the value of E_h when the ratio (oxidant)/(reductant) is 1 and when the activity of the hydrogen ion is 1, or the pH is zero. If the solution of the system under investigation were prepared in a solution of pH 0, and if a hydrogen electrode could be used in the same solution, then it would be possible to obtain E_h and E_0 directly from a cell without liquid junction, one electrode being bright platinum and the other platinized platinum saturated with hydrogen at 1 atmosphere pressure. Actually such a cell is practically identical with

[4] Paul Ehrlich (1854–1915), Director of the Prussian Institute for Serum Research and Serum Testing, Berlin, and later Director of the Georg Speyer Haus, Frankfurt am Main, was widely known for his work in chemotherapy, particularly the discovery of salvarsan or arsphenamine.

one in which the hydrogen electrode is immersed in a solution of the desired pH, but without any of the organic components of the oxidation-reduction system. In this way the effect of hydrogen on the equilibrium of the system is avoided, and the only liquid junction is that between two solutions of the same pH, identical except for the presence of the organic components in one solution only. Since the organic system will give stable potentials when present in very low concentrations and since it furnishes very few ions to the solution, the liquid junction potential must be very close to zero. Such a cell is theoretically much closer to the thermodynamic ideal of a reversible cell than any cell involving a junction with a potassium chloride solution.

Experimentally it is not necessary that such measurements be made in a solution of pH 0. If the organic components are dissolved in a solution of a strong acid, or in a buffer mixture, provided only that the pH is within the range in which equation (2) applies, the observed potentials will be identical with those at pH 0 if the measured potential difference is that between an inert electrode in this solution and a hydrogen electrode in a solution of the same acid or buffer without the organic components. This follows from the fact that the last term of equation (2) is identical with the Nernst formula for a hydrogen electrode concentration cell, one of whose electrodes is a normal hydrogen electrode.

Values of E_0 for many organic systems have been determined by Clark, Conant, Fieser, and their collaborators (27, 32). The direct method, without the use of potassium chloride junctions, is described by Conant and Fieser.

The Quinhydrone Electrode. The use of the quinhydrone electrode for pH measurements, as described in Chapter VI, is possible because equation (2) applies to the system quinone-hydroquinone. Quinhydrone is an addition compound of equal numbers of moles of quinone and hydroquinone, and this compound is partially dissociated into these components in solution. The addition of a little quinhydrone to any solution is therefore a simple method of ensuring that the ratio of the concentra-

tions of quinone and hydroquinone in the solution shall be 1. If the solution is sufficiently acid (pH less than about 8), equation (2) applies with the omission of the second term on the right, since

$$\log \frac{(C_6H_4O_2)}{(C_6H_4(OH)_2)} = 0.$$

If two quinhydrone electrodes are used with a potassium chloride bridge, the E.M.F. is related to the hydrogen ion activity by the same formula which applies to a cell with two hydrogen electrodes,

$$E = 0.0001984\ T \log \frac{a_H'}{a_H''}, \qquad (3)$$

which is identical with equation (8) of Chapter VI. Here, as before, the single prime refers to the solution of greater acidity if the E.M.F. as used in the equation is always given a positive sign. This equation may obviously be rewritten in terms of pH as

$$pH'' - pH' = \frac{E}{0.0001984\ T}. \qquad (4)$$

In equation (4), as in (3), E is always to be written as positive if the single prime refers to the more acid solution, which may be either the unknown or the standard solution. As in the case of pH determinations by the hydrogen electrode, it is necessary to note the actual polarity of the cell measured before applying the formula to calculate pH. If the electrode in the unknown solution is negative with respect to that in the standard, then the unknown has the greater pH, and vice versa. If the calomel cell is used with a single quinhydrone electrode, the latter is usually positive in a solution of any pH value likely to be met in practice. In such a case E in equation (3) or (4) is the difference between the E.M.F. values observed against the same calomel cell with the quinhydrone electrode in the standard and in the unknown solution, and the more acid of the two solutions is that giving the greater E.M.F.

The quinhydrone electrode was first used for pH measurements by Biilmann (16). He verified the parallel variation with pH of quinhydrone electrode potentials and hydrogen electrode potentials which is predicted by equation (2). In the presence of high concentrations of salt this parallelism is no longer exact. By an ingenious application of physico-chemical theory, electrodes have been devised (16, 111) which avoid this "salt error." These are the quino-quinhydrone electrode, consisting of an inert electrode in a solution saturated with both quinone and quinhydrone, and the hydro-quinhydrone electrode, in which the solution is saturated with both hydroquinone and quinhydrone.

The application of the quinhydrone electrode to pH measurements in blood serum was mentioned in Chapter VI (34).

Phase Boundary Potentials. The interface between two immiscible liquids, each containing the same electrolyte in solution, is, in general, the seat of a potential difference. This may be predicted by the following argument, adapted from Michaelis (81). Imagine that an aqueous solution of hydrochloric acid is shaken up with an oil immiscible with water; the acid will distribute itself between the two solvents. After the two phases have separated, let the solutions be poured into a U-tube so that the aqueous phase fills one limb and the oily phase the other. Now let a hydrogen electrode be immersed in each solution, and the E.M.F. between them be measured. The value of this total E.M.F. must be zero if the hydrogen electrode works reversibly in each solvent, since the system is in equilibrium. Since the acid is, in general, very unequally distributed between the two phases, the electrode potentials will not be equal (unless the constants in the equations for electrode potentials in the two solvents happen to differ just enough to cancel the effects of the different ion activities). Hence there must be between the electrodes a third potential difference, which is at the phase boundary.

Such a single phase boundary potential cannot be measured directly with calomel electrodes, as was done by Loeb in the case of the Donnan membrane potentials, because there would

be another phase boundary at the junction of the aqueous potassium chloride solution connecting the calomel electrode with the oily phase. However, Beutner (14) found it possible to measure the difference between two phase boundary potentials by setting up such cells as the following:

Calomel electrode	Na oleate in H_2O	Na oleate in oil	NaCl in oil	NaCl in H_2O	Calomel electrode
		1	2	3	

The double lines indicate negligible liquid junction potentials between aqueous potassium chloride and the other aqueous solutions. The vertical line marked 2 is also the seat of an ordinary liquid junction potential, which is likely to be of low magnitude, since the same solvent is on both sides of this boundary. The boundaries marked 1 and 3 are the seat of larger phase boundary potentials, since here the solvent is different on each side. The measured E.M.F. is thus not far from the difference between the phase boundary potentials at 1 and 3. Beutner studied a number of similar cells, and found surprisingly high values of the E.M.F., which was in many cases of the order of 0.1 volt. It is characteristic of all such cells that they are not in equilibrium, as may be inferred from the composition of the different phases in the diagram. Hence the values of the E.M.F. in such systems cannot, in general, be calculated from formulas derived by thermodynamics. Such cells may be of considerable importance, however, as models of bioelectric potentials, because of the considerable magnitude of the E.M.F. observed, the absence of metal electrodes, and the fact that non-aqueous lipoid phases are known to exist in living cells.

Electrokinetic Phenomena. This term includes cataphoresis, which is the migration of colloidal or suspended particles in an electric field, and electro-endosmosis. The latter term means the movement of a solvent or solution through a fixed membrane or diaphragm when a potential difference is imposed from an outside source on two electrodes in the liquid on opposite sides of the diaphragm. The explanation of the two kinds of motion is

the same; given a potential difference between solid and liquid, an outside electric field must tend to cause motion of one phase relative to the other. Cataphoresis occurs when the solid is mobile and the liquid is relatively motionless; electro-endosmosis, when the solid is held rigidly and the liquid is free to move.

The facts of electrokinetic behavior imply only that there must be a potential difference between two layers which move relatively to each other; they give no information as to the cause of such a potential difference. The potential difference may be due to selective adsorption of ions or to surface ionization of the solid material, or it may be, at least in part, of the nature of Donnan's membrane potentials or Beutner's phase boundary potentials. It is important that all of these possibilities be considered in attempting an explanation of electrokinetic behavior, as well as of the potential differences observed in living tissues such as muscle and nerve.

PROBLEMS

1. Certain living cells are found completely to decolorize (reduce) the dye methylene blue. If a half reduced solution of this dye has zero oxidation potential at pH 7.3, and if the cell contents have this pH value, what is the sign of E_h for the cell contents?

2. At 25°C. a quinhydrone electrode is 0.699 volt more positive than a hydrogen electrode in a solution of the same acidity. Plot a curve showing the relation of E_h to pH for the quinhydrone electrode from pH 1 to 8.

REFERENCES

26. CLARK, Chapters XVIII, XIX.
28. CLARK.
31. CONANT.[5]
46. GILLESPIE, Chapter XXVI.
52. HAMMETT, Chapters VII, VIII.
81. MICHAELIS, Chapters VI–X.
83. MICHAELIS.

[5] James Bryant Conant (1893–), President of Harvard University and Professor of Organic Chemistry, has been particularly successful in applying the methods of physical chemistry to problems of oxidation and reduction in organic systems.

CHAPTER XII

Transformations of Energy

Mechanical Equivalent of Heat. About the year 1798, in observing the boring of cannon in an arsenal in Munich, Rumford[1] noticed that the heat produced was roughly proportional to the work done by the horse whose power was used to run the drill. Another indication that work could be transformed into heat was obtained by Davy,[2] who noticed that two pieces of ice could be made to melt by rubbing them together. The principle of the equivalence of heat and work was first stated in 1842 by Mayer,[3] who formulated the law that when heat is produced from work, the heat obtained bears a constant ratio to the work done, and the same ratio holds when work is obtained from heat. Since work is the product of a force and a distance, it may be expressed in foot-pounds, kilogram-meters, gram-centimeters, etc. The unit of work in the c.g.s. system is the erg, which is the work done by a force of one dyne acting on a body which moves through a distance of one centimeter. Heat is usually measured in calories, one calorie (abbreviated cal.) being the heat required to raise the temperature of 1 gram of water from 14.5° to 15.5°C. (This is the 15° calorie, which is not very different from the mean calorie, or 1/100 of the heat required to raise 1 g. of water

[1] Benjamin Thompson (1753–1814) was born in New England, served in the British army during the American Revolution, and was later made a count, Graf von Rumford, by the Elector of Bavaria. His experiments on the production of heat by friction were among the earliest pieces of evidence against the caloric theory, according to which heat was a substance. He founded the Royal Institution in London.

[2] Humphry Davy (1778–1829) began his scientific career as an apprentice to a surgeon and apothecary. Later, as a result of his chemical researches, he became Professor of Chemistry of the Royal Institution in London. His name is associated with numerous chemical discoveries such as the isolation of sodium by electrolysis and the invention of the miner's safety lamp.

[3] Julius Robert Mayer (1814–1878), a German physician, is said to have become interested in heat and energy as a result of speculations as to the more intense red color of the venous blood of persons living in the tropics.

from 0° to 100°C.) In physiological work it is customary to use the large calorie (abbreviated Cal.), which is equal to 1000 small calories.

Mayer's principle was verified experimentally by Joule,[4] who determined the heat produced by a known amount of mechanical work in compressing air, stirring water with paddle wheels, rubbing two iron wheels together under mercury, forcing water through narrow holes, and rotating a coil of wire between the poles of a magnet, the electrical energy so produced being quantitatively converted into heat by the electrical resistance of the wire. These and other methods all gave approximately the same ratio of work to heat, and subsequent work has confirmed the equivalence more exactly. The best value for the mechanical equivalent of heat is 4.182×10^7 ergs per small 15° calorie. Joule's labors in this field have been commemorated by the use of his name for a unit of work, the joule being equal to 10^7 ergs; 1 calorie = 4.182 joules. The electrical units have been so defined that the work done by one coulomb of electricity flowing under an electromotive force of one volt is equal to one joule (in referring to electrical work, the joule is sometimes called the volt-coulomb).

Conservation of Energy. In 1847 Helmholtz stated the principle of the conservation of energy. It had been realized for many years that it was not possible to produce perpetual motion; that is, no machine can yield more work than is done upon it. Helmholtz recognized the connection between the impossibility of such perpetual motion and the equivalence of heat and work. We now refer to heat as a form of energy. Energy is defined as the capacity to do work; hence energy and work are measured in the same units. Helmholtz's principle may be stated as follows: *in all processes which take place in an isolated system, the energy of the system remains constant.* This statement, which is often called the first law of thermodynamics, is a

[4] James Prescott Joule (1818–1889) was an English brewer who became famous because of his researches on the mechanical equivalent of heat. He made many discoveries concerning heat, magnetism and electricity.

generalization from universal experience. It is one of the most fundamental laws in science.

The first law implies that the energy of a body is determined only by its state; that is, its temperature, volume, pressure, electrical condition, etc. We have no means of knowing the absolute value of the total energy of a body; energy measurements are concerned only with changes in energy as a body or system of bodies passes from one state to another. The first law says that the difference in the energy possessed by a body in two states is determined only by the conditions specifying these two states, and is independent of the path by which the body gets from one state to another. For example, in changing a gas from room temperature and atmospheric pressure to the standard conditions, we may change the temperature first, keeping the pressure constant, and then change the pressure, keeping the temperature constant, or we may reverse this order, or we may change both continuously; the energy change is the same in all cases. Likewise it takes the same energy to change a given mass of ice, say at $-10°$C., to steam, say at $110°$C., whether the ice is first melted to liquid water and the water boiled to form steam, or the ice is vaporized directly without passing through the liquid state and the vapor then heated to the required temperature.

If electrical and magnetic processes are excluded, the state of a body is determined by its pressure, volume and temperature. An equation relating the values of these three variables is often called an equation of state (or of condition). The most familiar equation of state is that for a perfect gas,

$$pv = NRT.$$

If a system passes from one state to another, the first law may be expressed by the equation

$$-\Delta U = Q + W.$$

Here $-\Delta U$ is the decrease in the energy content of the system, $+Q$ is the heat given up by the system to the surroundings, and $+W$ is the work done by the system on the surroundings.[5]

[5] Other conventions are in use as to the algebraic sign of energy changes.

Heat of Reaction. Since work is the product of a force and a distance, it is also the product of a pressure and a change in volume. If a gas expands by pushing a piston against the constant pressure of the atmosphere, the force acting is the pressure (force per unit area) times the area of the piston, and the distance through which this force acts is the change in volume divided by the area of the piston. For a change in state involving a volume change,

$$-\Delta U = Q_p + p\Delta v$$

where Δv is an increase in volume, $+p\Delta v$ is work done by the system, and Q_p is the heat evolved at constant pressure.

If the change in state takes place without change in volume, then no mechanical work is done. Although the pressure, and hence the force exerted on the walls of the container, may increase tremendously, yet no work is done because this force is not accompanied by motion through a distance. Under such conditions $W = 0$ and

$$-\Delta U = Q_v$$

or the decrease in energy of the system is equal to the heat evolved at constant volume.

For reactions in which the initial and final substances are all solids and liquids, the changes in volume, even at constant pressure, are usually small, and the work done is negligible in comparison to the heat evolved. For this case Q_p and Q_v are approximately identical. For reactions involving gases at constant pressure the work done is not, in general, negligible. For this case the relation between Q_p and Q_v may be calculated, with sufficient approximation, by assuming the gases to behave as perfect gases. One of the criteria of a perfect gas is that its energy content depends only on the temperature and not on pressure or volume. Hence for a reaction involving gases $-\Delta U$ may be considered to be identical in the expressions for Q_p and Q_v, and therefore

$$Q_v = Q_p + p\Delta v.$$

The quantity Δv, at constant temperature and pressure, depends only on the change in the number of moles of gas in the

system resulting from the reaction. If the perfect gas law applies,

$$Q_v = Q_p + (N_2 - N_1)RT$$

where N_1 and N_2 are the initial and final numbers of moles of gas in the system.

It follows from the preceding equations that both Q_p and Q_v are determined only by the initial and final states of the system, being independent of the path by which the change is effected. Thus it is a consequence of the first law that heats of reaction are algebraically additive, or that the heat of formation of a compound is the same whether the compound is formed directly from the elements, or indirectly in steps by any chain of reactions. This fundamental principle of thermochemistry is the law of Hess,[6] which was discovered in 1840, before the first law of thermodynamics had been formulated. This law is the basis of the use of heats of combustion of foodstuffs, as determined by burning in oxygen in a bomb calorimeter, in calculations of their heat value when burned in the animal body. It is to be noted that in all such calculations it is necessary to specify exactly the initial and final states of all substances concerned.

Heats of reaction are usually tabulated as values of Q_p, rather than Q_v, although in many cases, as in combustions, the reaction is actually carried out at constant volume. The calculation of Q_p from Q_v has already been considered. The heat of reaction, in general, varies with the temperature at which the reaction takes place. Moreover, heats of reaction are usually stated for reactions at constant temperatures. Actually the temperatures must vary, for heat is measured in a calorimeter only by the temperature change it produces. The procedure used is somewhat as follows. The change in state is allowed to take place in a calorimeter so insulated as to lose little or no heat to the surroundings, and the change in temperature in the calorimeter is exactly measured. Then an accurate determination is made of the quantity of heat which must be withdrawn from or added to the

[6] Germain Henri Hess (1802–1850), who was born in Geneva, became Professor of Chemistry at the University in St. Petersburg, Russia. He is known chiefly for these early researches in thermochemistry.

calorimeter and its contents to restore them to the initial temperature. It is this quantity of heat which is taken as the heat of reaction at a constant temperature equal to the initial temperature.

Heat of Ionic Reactions. Long before the theory of electrolytic dissociation had been formulated, Hess discovered a principle called the thermoneutrality of salt solutions. This is the fact that when two dilute salt solutions are mixed there is neither evolution nor absorption of heat. The explanation follows at once from the theory of complete dissociation of strong electrolytes. Thus if dilute solutions of sodium nitrate and potassium chloride are mixed, there can be no chemical reaction or other change of state, and hence no heat effect, because the salts are already fully ionized in solution. This rule fails to hold for solutions of those few salts which appear, from conductivity or E.M.F. data, not to be strong electrolytes.

Hess also discovered that when dilute solutions of strong acids and bases are mixed in equivalent amounts the heat evolved is independent of the chemical nature of the acid and base. This constant heat of neutralization is about 13,800 cal. at 18° for the reaction between 1 equivalent of acid and base, the solutions being 0.12 to 0.25 normal. This again is a consequence of the theory of complete ionization, the reaction being in each case

$$H^+ + OH^- \rightarrow H_2O.$$

Conversely the heat absorbed on the complete ionization of 1 mole of water must also be 13,800 cal.

When either the acid or base is a weak electrolyte, the heat evolved on neutralization may be either greater or less than 13,800 cal., according as the further ionization of the weak electrolyte is an exothermic (giving off heat) or endothermic (absorbing heat) reaction. Such heat effects have proved to be important in attempts to account for the heat of muscular contraction. Here a fairly strong acid, lactic acid, is believed to be formed, but much of its hydrogen ion is immediately combined with bicarbonate ion or negative protein ions to form weaker acids, carbonic acid and un-ionized protein.

Free Energy. Any system not in equilibrium can be made to do work. The maximum work which such a system can produce is called the change in free or available energy accompanying the reaction. This free energy decrease is not, in general, equal to maximum heat obtainable from a reaction; it is usually less, though in some cases it may be greater if the production of work by the system involves the abstraction of heat from the surroundings. The free energy decrease of a reaction, and not the heat obtainable from it, is a measure of the driving force of the reaction; from a practical point of view it is far more important to know the maximum work than the maximum heat obtainable from a reaction. Free energies are additive, just as are heats of reaction. One of the most important recent advances in chemistry has been the construction of free energy tables which make it possible to predict the extent of a chemical reaction under untried conditions (75).

The use of the free energy concept in physiology is only just beginning; for its intelligent use, one must obtain some mastery of the principles of thermodynamics. It is desired here to emphasize only that there is such a concept, much more fundamental than that of heat of reaction, and that progress in the study of physiological transformations of energy is likely to be made by those who are somewhat familiar with the science of the transformations of energy, which is thermodynamics.

PROBLEMS

1. The heat of combustion of 1 mole of sucrose at 18°C. is given as $Q_v = 1{,}349{,}600$ cal. Calculate Q_p for this substance. What is the connection between the respiratory quotient (defined in text books of physiology) and the relation between Q_v and Q_p for the substances oxidized in respiration?

2. If the heat of formation of liquid water from the elements is 68,400 cal. per mole, and that for CO_2 is 94,400, calculate the heat of formation of sucrose, using its heat of combustion from Problem 1.

3. If the heat of combustion of glucose is 673,000 cal. per mole, and that of glycogen is 4,190 cal. per gram, calculate the

heat change accompanying the formation of one mole of glucose from glycogen.

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46. GILLESPIE, Chapters XV, XIX.
75. LEWIS and RANDALL, Chapters V, XIV, XV.
97. NOYES and SHERRILL, pp. 35–40, 207–229.
127. WASHBURN, Chapters X, XIX.

LABORATORY DIRECTIONS

EXPERIMENT 1

Osmotic Pressure and Cell Volume

This experiment is best done by the instructor as a demonstration. The object of the experiment is to study the effects of solutions of sodium chloride of varied concentration on the volume of red blood cells, and to determine that concentration which is isotonic with the cell contents and the serum in which the cells are suspended. The method is a modification of the hematocrit method, first used by Hedin (58) and Köppe (68). The hematocrit is an instrument for determining the percentage volume occupied by the red cells in blood, or in blood diluted with some solution, by measuring the height of a uniform column of cells after centrifuging. Various types of hematocrits have been described by Hamburger (51) and others (119, 131).

The apparatus consists of a U-tube of uniform capillary tubing with an open bulb sealed on each end (Fig. 27). The U-tubes are calibrated by measuring the length of a weighed amount of mercury in the straight part of each arm of the tube, and the apparent height of the ends of the same column of mercury when it occupies the bend of the tube. This height is measured from the outer circumference of the bent part of the tube, by placing the tube vertically on an L-shaped block of wood provided with a vertical scale graduated in millimeters. This calibration makes it possible to get the length of a column of blood cells, in terms of the length it would have in the straight part of the tube, while the column is occupying the bend of the tube after centrifuging.

The tubes are filled with suitable amounts of defibrinated blood measured by a graduated pipette with a capillary tip. The amounts used are determined from the calibration of the tubes, and the known average percentage of red cells in blood, so that, after centrifuging, the top of the column of cells in each

arm of the tube lies 1 or 2 cm. below the bulb. Each tube is then supported in a split wooden cylinder having grooves to fit the arms of the tube, and placed in a 50 ml. brass centrifuge cup provided with the usual rubber cushion at the bottom. The tubes are centrifuged for 20 minutes at about 3400 revolutions per minute. This does not give quite the minimum attainable volume of the cells, but is within a few per cent of it. The height of the cells in each arm is measured with the L-shaped block,

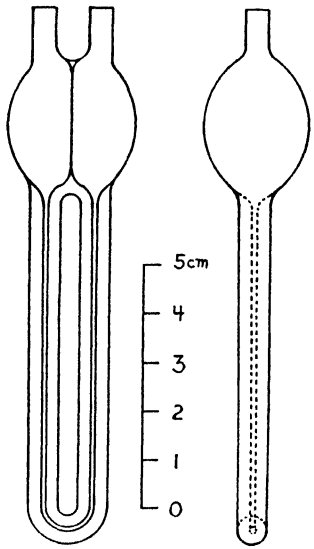

FIG. 27. Hematocrit tube*

and the heights are added for each tube. Then a measured amount of sodium chloride solution, equal to 12.5 times the volume of the blood, is put into each tube, and mixed with the blood by sucking the cells back and forth 8 times from one bulb to the other. The concentrations of salt used are 0.8, 0.9, 1.0 and 1.1 grams per 100 ml. The mixtures are allowed to stand for 20 minutes to ensure the attainment of osmotic equilibrium, and centrifuged again for the same time at the same speed. The new heights of the columns of cells are read, and the changes are determined by difference. These are converted into percentage

* These tubes were designed with the help of Dr. L. F. Nims, and made by F. Pierce Noble, Glassblower, Sterling Chemistry Laboratory, Yale University.

changes in volume by multiplying by 100 and dividing, not by the heights measured with the L-shaped block, but by the true lengths of the columns of cells in the defibrinated blood, obtained by correcting the original readings by means of the calibration data. The percentage changes in volume are plotted against the concentrations of the added salt solutions, and a smooth curve, usually almost a straight line, is drawn through the points. The salt concentration corresponding to zero volume change is read from the curve, and should be that of a solution isotonic with the cells or serum of the blood sample used.

This method rests on the assumptions that the cells are impermeable to sodium chloride, that the volume of the cells changes only as a result of the osmosis of water, and that the technique employed gives a true measure of the relative changes in cell volume. Some of these assumptions are open to question, but still the method gives a result for the isotonic concentration which is in the range of the usually accepted values.This method is more accurate than many other hematocrit methods because it does not require an accurate measurement of the total volume of blood used in each tube. The experiment illustrates, among other things, the possible error in taking hematocrit readings with blood treated with oxalate, or some other anticoagulant salt, as a measure of the cell volume in whole blood.

This experiment may also be done with the hematocrit tubes of Van Allen (119) or of Wintrobe (131), which are on the market. The former tubes permit a high dilution of blood with the salt solutions, but the latter, because of the absence of a bulb, permit only a mixture of equal parts of blood and solution. In order to obtain sufficient volume change to be measured in the Wintrobe tubes, it is well to use a wider range of salt concentrations, such as 0.5, 0.8, 1.1 and 1.4 g. per 100 ml. In using these tubes it is necessary to determine the normal volume of the cells by a control experiment with undiluted blood, or with blood diluted with an equal volume of its own serum.

REFERENCES

51. HAMBURGER, pp. 379, 385, 515.
58. HEDIN.
67. HÖBER, p. 421.
68. KÖPPE.

EXPERIMENT 2

Osmotic Hemolysis

This is a modification of an experiment of Hamburger, by which he showed in 1883 that the least dilute aqueous solutions of certain salts or sugars which cause the coloring matter to leave red blood cells have about the same osmotic pressure (but not the same as the cell contents). This work was the first application of physical chemistry to physiological material of animal origin, and Hamburger's results served as confirmatory evidence for the theories of osmotic pressure and electrolytic dissociation of van't Hoff and Arrhenius.

The experiment is best done by several students working with solutions of different substances. Starting with a stock solution of each substance, a series of concentrations is prepared by accurate dilution with water. For accuracy in dilution it is well to prepare 10 ml. of each concentration, although only 2 ml. are required for the experiment. The 2 ml. samples are placed in test tubes about 13 × 100 mm. in size. The substances used, and their concentrations in g. per 100 ml., are as follows:

Sodium chloride	0.40	.45	.50	.55	.60	.65	.70	.75
Potassium nitrate	0.80	.85	.90	.95	1.00	1.05	1.10	1.15
Sodium sulphate	0.70	.75	.80	.85	.90	.95	1.00	1.05
Sucrose	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0
Ammonium chloride	0.4	.6	.8	1.0				
Urea	0.5	1.0	1.5	2.0				

To each 2 ml. sample of solution, one drop of defibrinated blood is added, and the tubes are gently shaken and allowed to stand for 15 minutes with occasional shaking. Any tubes which still look cloudy at the end of that period, indicating the presence of intact blood cells, are then gently centrifuged for 5 minutes at about ¼ of the full speed of the centrifuge. Before centrifuging, each tube is placed in a brass centrifuge cup having a rubber cushion in the bottom. The tubes and cups are balanced in pairs to 0.1 g. by putting water in the cups outside of the glass tubes, and each pair of balanced tubes is placed on one diameter of the centrifuge head.

After centrifuging, the tubes are observed against a white background to determine the highest concentration in each series which has permitted the escape of the red coloring matter, hemoglobin, from the blood cells. Another way of comparing the effects is to note the concentrations of the different substances which produce the same depth of color in the supernatant liquid. These concentrations of the different substances, which show comparable effects on the red blood cells, are then translated into moles per liter, and the freezing points of these solutions are read from curves constructed from data in the *International Critical Tables* (87).

Before attempting to explain the results obtained with ammonium chloride and with urea, students should add a drop of blood to 2 ml. of a solution containing both ammonium chloride and sodium chloride, each being 0.15 *M*, and also to 2 ml. of a a solution which is 0.3 *M* in urea, and 0.15 *M* in sodium chloride. It is possible to explain both experiments on an osmotic basis, by assuming differences in the permeability of the cells to different solutes.

The results of the experiments with the first four substances may be compared with those of Hamburger (51, p. 237).

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51. HAMBURGER, pp. 164, 237, 378, 439.
67. HÖBER, pp. 419–422.
77. LUCKÉ and MCCUTCHEON, pp. 68–83.
114. STARLING, pp. 82, 700.

EXPERIMENT 3

DETERMINATION OF FREEZING POINTS

The object of this experiment is to measure, within a few thousandths of a degree Centrigrade, the freezing points of a series of sodium chloride solutions of varied concentration, including the range of physiological salt solutions. Freezing points have often been determined in physiological laboratories by the Beckmann method, which is sufficiently exact for the approximate determination of molecular weight of dissolved sub-

stances. Since that method uses a cooling bath several degrees below the desired freezing point, it is difficult to obtain by it a true equilibrium condition. Recent precise determinations of the freezing points of aqueous solutions (105) have been based on a different principle. This consists in bringing a large quantity of crushed ice into equilibrium with the solution in a well insulated vessel, and determining the temperature and the composition of the solution as accurately as possible. In work of extreme precision, temperature is measured by thermo-elements. A method of moderate precision, suitable for biological liquids containing proteins, has been described by Stadie and Sunderman (113). The present method resembles theirs in the use of a mercury thermometer, but resembles the more precise methods in that larger quantities of ice and solution are used, the composition of the solution being determined by analysis.

The freezing point vessel is an ordinary pint vacuum bottle. The thermometer is either a Heidenhain thermometer with fixed zero point or a Beckmann thermometer with an adjustable zero point. Students using the Beckmann thermometer may need assistance in setting the zero point. Either type has a scale graduated in hundredths of a Centigrade degree, so that thousandths may be estimated by the use of a magnifying glass. The thermometer is supported in the bottle by a rubber stopper, containing also a hole for the handle of the stirrer (which is a glass rod with a ring at the bottom) and another hole for a pipette used to remove samples of solution. It is necessary to determine the zero point of either thermometer by determining the freezing point of pure water.

The vacuum bottle is about half filled with finely crushed ice, preferably frozen from distilled water. Commercial ice may be used if superficially washed with cold distilled water after crushing. About 100 ml. of distilled water, previously cooled to 0°C., are added, the stopper is put in place, and the apparatus left at room temperature. For more exact work it is well to submerge the vacuum bottle, without its case, in a pail of crushed ice and water. The stirrer is moved only a few times just before each reading is taken, and readings are taken at 5 minute inter-

vals. When 4 such readings agree within 0.003°C. their average may be taken as the equilibrium temperature or the freezing point of water.

About 14 ml. of 10 per cent sodium chloride solution are then added and mixed with the ice and water by shaking and stirring. A dry 10 ml. pipette is inserted through the stopper and the apparatus is left undisturbed as before, with observations at 5 minute intervals. After the temperature has become constant a sample of 10 ml. is removed and put into a stoppered flask. If the freezing point is as low as −0.65°C., the remaining mixture is diluted with 5 or 10 ml. of water at room temperature, the bottle is shaken, and another set of readings and a sample are taken. If the freezing point is not so low as −0.65°, the solution is concentrated by adding a little more 10 per cent salt solution. In this way 4 or 5 samples should be obtained of solutions having freezing points in the range −0.65 to −0.45°C. In exact work, the amount of solution in each sample may be determined by weighing the flask and its contents to the nearest 5 mg. and obtaining also the weight of the empty flask. After adding 1 drop of a saturated solution of potassium chromate, the amount of sodium chloride in each sample is determined by titration with standard 0.1 *M* silver nitrate solution, the end point being a color change from yellow to reddish orange. The freezing point depressions are plotted against the molal concentrations of sodium chloride. Over this short range the points should fall quite exactly on a straight line. If this is the case, the equation of the line should be determined from the data. The accuracy of the results may be checked by comparison with the data of Scatchard and Prentiss (106).

The freezing point of mammalian serum is usually given as −0.56°C. ±0.02°, and the solution of sodium chloride having this freezing point should be isotonic with serum. For an experimental determination of the freezing point of serum, the smaller scale apparatus of Stadie and Sunderman (113) may be recommended.

REFERENCES

105. SCATCHARD, JONES and PRENTISS.
113. STADIE and SUNDERMAN.

EXPERIMENT 4

Hydrogen Ions and Buffers

Buffer Solutions. The following mixtures are prepared in 4 test tubes:

Tube No.	1	2	3	4
ml. 1.0 *M* sodium acetate	1	1	1	1
ml. 0.25 *M* acetic acid	4	2	1	0.5
ml. distilled water	6	8	9	9.5

After mixing the contents of each tube, 1 ml. is removed from each and added to 9 ml. of distilled water, these dilute solutions being in a second series of 4 tubes. Into each of the 8 tubes are put 5 drops of methyl red indicator solution, the contents of each tube being mixed again. Any difference in the colors of the indicator in corresponding tubes of the two series should be noted. That this result is a property of a buffer solution may be shown by making a similar dilution of 0.1 *M* sodium chloride in 0.001 *M* hydrochloric acid, which is not a buffer solution. In this case a suitable indicator would be methyl orange or brom phenol blue.

The different resistance of buffered and unbuffered solutions to changes in acidity may be shown by preparing a mixture of 10 ml. of 1.0 *M* acetic acid, 1 ml. of 1.0 *M* sodium acetate, and 3 drops of methyl orange solution. In another test tube are placed 10 ml. of 0.1 *M* sodium chloride and 3 drops of methyl orange. To the latter mixture, 0.01 *M* hydrochloric acid is added, drop by drop, with stirring, until the color matches that in the first tube. When this is the case, the two solutions may be said to have the same concentration of hydrogen ions. Their resistance to changes in acidity is tested by adding to each mixture 0.5 ml. of a 1 per cent gelatin solution (or of any other dilute solution of a substance capable of combining with hydrogen ions). Students should be able to explain the resulting colors, and to give a reason for the higher buffering capacity of one of the mixtures.

Range of Different Indicators. The fact that each indicator is useful only over a limited range of acidity may be shown by preparing 2 more sets of 4 buffer mixtures 0.1 *M* in sodium acetate, as in the first part of the preceding section, and test-

ing them with other indicators, such as brom phenol blue and brom cresol purple.

Colorimetric Determination of pH. The pH of an unknown solution is determined by adding indicator and matching the color with that of one of the standards of known pH. By preliminary tests with a single drop of indicator and a drop or two of solution, an indicator is selected to which the solution gives a color in the useful range of the indicator. In the final comparison the volume of solution and the amount of indicator used must be identical for the unknown and the standard solutions, 5 drops of indicator being added to 10 ml. of solution. In case the unknown solution is colored, the comparison is made by looking through two pairs of tubes at once in a comparator block. Light reaches the eye through a standard tube with indicator and a tube of the colored unknown solution without indicator, and again through a tube of the colored unknown solution with indicator and a tube of water.

The experiments with buffers have been adapted from Michaelis (82). This colorimetric method of pH determination was introduced into biochemistry by Sørensen (110). The idea of the comparator for colored solutions was used by Walpole (126). The theory and practice of pH determinations are discussed at length by Clark (26).

REFERENCES

26. CLARK, Chapters I–III.
81. MICHAELIS, Chapters I, II.
82. MICHAELIS, Experiments 14, 15, 20.

EXPERIMENT 5

IONIZATION CONSTANT OF AN INDICATOR; DETERMINATION OF pH WITHOUT BUFFERS

Ionization Constant of an Indicator. By having a given amount of indicator distributed between two tubes, one containing an excess of acid and the other an excess of alkali, and looking through both tubes, one behind the other, it is possible to obtain a color which matches that produced by adding the same total amount of indicator to a suitable buffer solution in

a single tube. In each case it may be assumed that the same fraction of the total indicator is in the ionized form. For this purpose most indicators may be considered to behave as weak acids, and the fraction of the total indicator in the ionized or alkaline form is given by the ratio of the number of drops of indicator in the alkaline tube to the total number of drops in both tubes.

The indicator to be used in this experiment is either methyl red, brom thymol blue, brom cresol purple, or phenol red. The same buffer standards may be used as in Experiment 4; these have 5 drops of indicator added to 10 ml. of buffer. The concentrations of the indicator solutions used in making up these standards are 0.02 per cent for methyl red and phenol red, and 0.04 per cent for the others. In this experiment, indicator solutions of just half these concentrations are used, a total of 10 drops being added to 2 tubes, each containing 10 ml. of solution. The test tubes used in this experiment, as in all colorimetric pH work, should be of uniform bore. This may be checked, if necessary, by putting 10 ml. of water into each tube, using only those tubes in which the height of the columns of water is the same within 2 or 3 mm. in about 50 mm.

A set of 9 tubes is prepared, each containing 10 ml. of water and 2 drops of 0.1 *M* hydrochloric acid, and a similar set containing 10 ml. of water and 2 drops of 0.1 *M* sodium hydroxide. If the number of tubes available is limited, only 2 or 3 pairs need be made up at one time. To the acid tubes are added, in order, 1, 2, 3, and so on up to 9 drops of indicator. To the alkaline tubes different amounts of indicator are added in the same way, each tube being marked. The tubes are viewed in pairs in a comparator block, in such a way that if x is the number of drops of indicator in the alkaline tube, the corresponding acid tube will have $10 - x$ drops of indicator. Then the fraction of the total indicator in the alkaline form will be $x/10$, and the ratio of that in the alkaline form to that in the acid form will be $x/(10 - x)$. Each pair of tubes should be matched with one of the buffered color standards of known pH, viewing the standard in the comparator through a tube of water.

The values of $x/10$ are plotted against the corresponding pH values of the standards. The logarithm of $x/(10 - x)$ is also plotted against pH, and a straight line is drawn through or near the points of the latter plot. According to theory this line should have a slope of 1, or it should be at an angle of 45° to the axes if the same scale is used for the logarithms and the pH values on the two axes.

From an inspection of these two plots of the results, it is possible to determine the value of pK, or the negative logarithm of the apparent dissociation constant, for the indicator used. A value of pK may also be calculated by the law of mass action from each point on these curves, or from the observation made with each pair of tubes.

Determination of pH without Buffers. By means of the theoretical mass law relation between pH, pK, and the logarithm of the ratio of drops of indicator, it is possible to use this method to determine the pH of an unknown solution, without the use of standard buffer solutions. It is of course necessary to know in advance the pK value for the indicator to be used, and to select the proper indicator so that the color given to it by the unknown solution will fall near the middle of its range of color gradations. The pH of an unknown solution should be determined in this way.

The method may also be used with slightly colored or turbid solutions, but in this case a comparator block is required with holes so bored that one may look through sets of 3 tubes instead of 2.

This drop method of determining pH without buffers was proposed by Gillespie (47), and a similar method applicable to one-color indicators was introduced by Michaelis and Gyemant (85). The Gillespie method has been refined by Hastings, Sendroy and Robson (57) who measured diluted indicator solution from a micro burette. These methods and the underlying theory are well presented by Clark (26).

REFERENCES

26. CLARK, Chapter VI.
47. GILLESPIE.
82. MICHAELIS, Experiment 18.

EXPERIMENT 6

SURFACE PHENOMENA

Measurement of Surface Tension. One of the most convenient methods for measuring surface tension consists in measuring the maximum pull on a platinum ring at the instant when it is detached from the surface of a liquid. A convenient piece of apparatus for this purpose has been described by Du Noüy (37), and the precautions and corrections necessary in using this method for the precise determination of absolute values of surface tension have been discussed by Harkins and Jordan (54).

In the present experiment the surface tension of several solutions, relative to that of pure water (72.8 dynes per cm. at 20°), is measured by the ring method. The instrument* used is a simple torsion balance, so constructed that the reading on a graduated dial is proportional to the pull exerted by the ring. The instrument is calibrated as follows: A small Petri dish is carefully cleaned and filled with about 5 ml. of distilled water. The dish is placed on the movable platform of the torsion balance, and the platform is adjusted so that the ring hangs over the center of the dish about 5 mm. above the surface of the liquid. The ring and the liquid must not be touched by the hands, as any contamination of the surface by oily matter has a tremendous influence on the surface tension. The ring is lowered by turning the screw at the back of the graduated dial until the ring is in contact with the liquid. The ring is then slowly raised by turning the screw in the opposite direction. It is important that the arm carrying the ring shall always be at the same level when the ring breaks away from the surface. This is accomplished by adjusting the screw controlling the height of the platform as well as the screw controlling the height of the ring. At the moment before the ring breaks away the top of the arm which supports the ring should be at the same level as the upper line on the mirror, the eye being so placed that the arm appears to be level with its image in the mirror as well as with the

* This is essentially the apparatus of Du Noüy (37) with slight differences in the arrangement of some of the adjustments.

line. After the platform has been adjusted so that this is the case, several readings are taken to the nearest fifth of a scale division, by raising the ring until it just breaks away from the surface. In repeating the measurement the platform is not moved, but the ring is lowered by turning the dial back until the ring drops into contact with the liquid. The measurement is repeated a few times with a second sample of distilled water. From the average of the readings of the dial, and the accepted value of the surface tension of pure water, a factor is calculated for the conversion of dial readings into values of surface tension in dynes per cm.

In order to study the variation of surface tension with the concentration of dissolved substances, the following solutions are prepared, and their surface tensions are measured by the same procedure used in the case of water.

Solution No.	1	2	3	4	5	6	7	8	9	10
ml. 25 per cent sodium chloride	2	4	6	8	10					
ml. 95 per cent ethyl alcohol						2	4	6	8	10
ml. distilled water	8	6	4	2	0	8	6	4	2	0

From the measurements and the calibration, the surface tension of these solutions is obtained in dynes per cm. The variation of these values with concentration is best exhibited by plotting the surface tension of the solutions of each substance against the mole fraction of that substance in the solution.

Certain materials of biological origin have a very great effect on the surface tension of water. This may be illustrated by measuring the surface tension of a 0.1 per cent solution of sodium taurocholate, which is one of the bile salts.

As exercises based on this experiment, students should derive a formula for calculating absolute values of surface tension from the maximum pull on the ring and its dimensions, and indicate how the torsion balance might be calibrated, without the use of a reference liquid, to give direct readings of the force exerted on the end of the arm.

The relations between surface tension and the concentration of aqueous solutions are discussed by Freundlich (44), and

examples of the importance of surface tension in systems of biological origin are given by Du Noüy (38).

Unimolecular Surface Films. A clean rectangular dish (5×7 photographic tray) is half filled with distilled water. A little powdered talc is dusted (by sifting through cloth) on to the surface. The surface is cleansed by gently blowing the talc to one end of the tray and pushing after it a barrier consisting of a strip of paper or metal. The barrier is left near the far end ofthe tray, with the talc and any oily impurities behind it. Another barrier is now put on the surface at the near end of the tray, and the clean surface between the two barriers is lightly dusted with talc. A known small amount of an oily or fatty substance is then put on this surface. This may conveniently be done by using a benzene solution; for example, 50 mg. of stearic acid in 100 ml. of benzene. Since the weight of the fatty substance added must be known, a 1 ml. pipette is calibrated with this solution by counting drops. About 0.08 ml. are delivered from this pipette on to the enclosed surface. The rear barrier is left fixed and the front one is pushed slowly towards it. Eventually the talc becomes pushed into a solid continuous film, and further compression produces wrinkles or ridges. Now the barrier is pulled slowly away, stretching the film, until a position is found beyond which the talc (which now presumably rests on a film of stearic acid) no longer follows the barrier as a continuous film. On the assumption that this critical position of the barrier defines the maximum area of a continuous film of stearic acid one molecule thick, the cross section of the stearic acid molecule (area of the surface occupied by it) is calculated from the dimensions of the enclosed surface, the weight of stearic acid added, its molecular weight, and Avogadro's number. Since the density of stearic acid is 0.84, the thickness of the film or the length of the molecule may also be calculated. The results should be compared with those of Langmuir (73).

REFERENCES

37. Du Noüy.
44. Freundlich, pp. 55, 61.
73. Langmuir, pp. 1858–1868.

EXPERIMENT 7

ADSORPTION

Effect of Solvent. About 10 ml. of a relatively concentrated (0.2 per cent) solution of a dye such as methyl orange are shaken with about 0.5 g. of animal charcoal. The mixture is filtered through ordinary filter paper. Under proper conditions the filtrate will be colorless. A clean test tube is placed under the funnel, and the latter is filled with alcohol or acetone. The color of this second filtrate illustrates the dependence of the completeness of adsorption on the nature of the solvent.

Effect of Concentration. A stock solution of 1.0 *M* acetic acid is accurately diluted to make 50 ml. of each of the following concentrations: 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, and 0.005 *M*. To attain the requisite accuracy in dilution, volumetric pipettes and flasks are used, and none of the volumes measured should be less than 5 ml. This will require dilution in several steps to make the more dilute solutions. To a 50 ml. sample of each solution is added 0.5 g. ($\pm$0.005 g.) of animal charcoal. The charcoal may be weighed with this accuracy on a "pulp" balance not protected by a case, the samples being weighed on tared pieces of glazed paper. The mixtures are shaken gently at intervals during half an hour. Each mixture is then filtered, the filtrate being received in a clean dry flask or beaker. The concentration of acetic acid remaining in each filtrate is determined by titrating an accurately measured sample with 0.1 *M* or 0.01 *M* sodium hydroxide, using phenolphthalein as indicator. In order to attain sufficient accuracy, the volume of the sodium hydroxide required should not be much less than 5 ml. This may be accomplished by using as much as 45 ml. of the more dilute acid filtrates, but only 5 ml. of the most concentrated. The initial concentrations of acid are obtained from the dilution factors of the original 1.0 *M* acid, which should be standardized against the 0.1 *M* sodium hydroxide. The results should be tabulated to show the volumes used in titration, the initial and final concentrations of acid, the changes in concentration, and

the number of moles of acetic acid removed from each solution per gram of charcoal. The latter quantity is plotted against the final concentration of acid left in solution, and a logarithmic plot of the same variables is also prepared.

If the results agree with Freundlich's adsorption formula or isotherm, the logarithmic plot should be a straight line. A different type of equation or isotherm was found by Langmuir to fit some adsorption data better than the equation of Freundlich. Both equations and most adsorption data are characterized by a rapid increase of the amount adsorbed with increase in concentration at low concentrations, while at higher concentrations the curves become much less steep.

REFERENCES

44. FREUNDLICH, pp. 111, 113, 172, 192.
74. LANGMUIR, pp. 1368–1371, 1384.

EXPERIMENT 8

OSMOTIC PRESSURE OF COLLOIDS

This experiment is best done by the instructor as a demonstration. Krogh (70) has devised a simple osmometer capable of giving reliable results for the colloidal osmotic pressure of blood serum. This instrument has the advantage that it requires only a small amount of serum; it is constructed so that the osmotic flow is counterbalanced by an external pressure. According to Krogh and Nakazawa the same results are obtained with either 0.9 per cent sodium chloride, Ringer's solution, or an ultrafiltrate from the serum itself as the external fluid.

Filter paper and flat membranes, which may be made from Cellophane No. 600 (118), collodion, or other material impermeable to dissolved proteins, are cut by a cork borer to fit the apparatus, and soaked in 0.9 per cent sodium chloride solution. The membrane is centered on two thicknesses of the filter paper and the three units are placed upon the silver disk with the membrane on top. The membrane is then carefully blotted with filter paper, and a drop of the salt solution is put upon the under

side of the silver disk. The osmometer is then assembled and filled with serum (obtained by centrifuging clotted or defibrinated blood) from a pipette so that no air bubbles remain in the upper chamber. The capillary tube is then inserted and adjusted so that the meniscus in the capillary tube is at a suitable point for observation. The lower part of the apparatus is submerged in a beaker of 0.9 per cent sodium chloride solution, and the upper end of the capillary tube is connected to a manometer. The pressure is increased slowly by raising the bulb connected to the manometer, keeping the meniscus in the capillary tube at the same level, until there is no tendency for the meniscus to rise. Equilibrium is reached in from four to six hours. The osmotic pressure in mm. of water is given by the difference in levels in the manometer tubes, plus the difference in levels of the meniscus in the capillary and the solution in the beaker, minus the height of the capillary rise of the serum under examination in the same capillary tube.

The principal advantages of this form of osmometer are the relative rapidity with which equilibrium is attained, the absence of concentration changes due to osmosis of water, and the small volume of liquid required. The principle of preventing osmosis by an external applied pressure was used by Berkeley and Hartley (13) in working with sugar solutions and by Sørensen (109) with the protein egg albumin. Other simpler forms of osmometers have been used for protein solutions by Loeb (76), Burk and Greenberg (23), and others. The results obtained with serum have been reviewed by Meyer (80).

REFERENCES

70. KROGH and NAKAZAWA.
80. MEYER.

EXPERIMENT 9

HYDROGEN IONS AND PROTEINS

Effect of Hydrogen Ion Concentration on the Swelling of Gelatin. In a series of 9 uniform test tubes are placed 0.2 g. samples of powdered gelatin, previously washed free from

electrolytic impurities by the method of Northrop and Kunitz (95). These samples may be taken by volume with a small measure, instead of by weighing, if care is used to ensure uniform packing of the powder. To each portion are added 25 ml. of one of the following solutions: 0.1, 0.01, 0.001 *M* hydrochloric acid; distilled water; 0.001, 0.01, 0.1 *M* sodium hydroxide; 0.01 *M* hydrochloric acid which is also 0.1 *M* in sodium chloride; 0.01 *M* sodium hydroxide which is also 0.1 *M* in sodium chloride. Each mixture is stirred until the gelatin is thoroughly wet, and the mixtures are allowed to stand at room temperature* for one or two hours, with occasional agitation. The different volumes occupied by equal weights of gelatin in the several solutions are roughly measured by noting the heights of the gelatin layers after the particles have settled. The results of similar experiments have been explained by Loeb (76).

Effect of Hydrogen Ion Concentration on the Solubility of Gelatin. About 1.5 g. of powdered ash-free gelatin are stirred into 15 ml. of distilled water, and allowed to soak for half an hour with occasional stirring to ensure thorough wetting. Meanwhile, the following series of acetate buffers 0.01 *M* in sodium acetate is prepared in 6 test tubes:

Approximate pH	4.1	4.4	4.7	5.0	5.3	5.6
ml. sodium acetate, 0.05 *M*	4	4	4	4	4	4
ml. acetic acid, 0.05 *M*	16	8	4	2	1	0.5
ml. distilled water	0	8	12	14	15	15.5

These solutions are divided into equal parts by transferring 10 ml. samples to a second series of 6 test tubes.

The gelatin is dissolved by warming the test tube under the warm water faucet, and mixed thoroughly to make a 10 per cent solution. A 1 per cent solution is also prepared by diluting 2 ml. of the 10 per cent solution with 18 ml. of distilled water.

One set of the acetate buffers is made about 0.9 per cent in gelatin by adding 1 ml. samples of the 10 per cent solution. The contents of each tube are mixed thoroughly while warm, and the

* A temperature of 15°C. is more satisfactory. If the temperature is much above 20° the gelatin dissolves in the more acid and more alkaline solutions.

tubes are placed in a refrigerator or other cool place. After cooling for at least an hour, or preferably over night, the tubes are examined for differences in turbidity or transparency, which should be noted and explained.

The second set of similar buffers is made about 0.09 per cent in gelatin by adding 1 ml. samples of the 1 per cent solution. The contents are thoroughly mixed, and the tubes are allowed to attain uniform temperature in the laboratory. To each solution 15 ml. of 95 per cent alcohol are added, the contents of the tubes are mixed, and the mixtures are left at room temperature for about half an hour. These tubes are likewise examined for differences in turbidity, which should be noted and explained.

In these experiments the only variable quantity is the pH or acidity of the mixtures. The results are therefore to be explained in terms of an effect of hydrogen ion concentration on some property of the protein. It should be possible to make a generalization as to the effect of pH on the solubility of proteins, or of amphoteric electrolytes in general.

The swelling of gelatin in acid and alkali was studied by Wo. Ostwald as early as 1905. The results of such experiments were first explained satisfactorily by Procter and Wilson in 1916. Their explanation, on the basis of Donnan's theory of membrane equilibria, was supported by the numerous experiments of Loeb (76).

The effect of acidity on the precipitation of gelatin by alcohol was studied by Pauli and Matula in 1913. Similar experiments with casein, without the addition of alcohol, had been done by Michaelis and Pechstein in 1912. The variations in the turbidity of gelatin gels with acidity were also studied by Pauli. For an idea of the most generally accepted explanations of such experiments, students are referred to the books of Michaelis, Loeb, and Pauli.

REFERENCES

76. LOEB, pp. 6–10, 44–47, 119–126, 240–258.
81. MICHAELIS, pp. 69–73, 86–87.
82. MICHAELIS, Experiments 49, 51, 28a.
98. PAULI and VALKÓ, pp. 83, 220, 229, 283.

EXPERIMENT 10

DETERMINATION OF AN ISOELECTRIC POINT BY CATAPHORESIS

A series of 4 acetate buffers is prepared according to the following table:

Approximate pH	4.3	4.6	4.9	5.2
ml. sodium acetate, 0.05 *M*	4	4	4	4
ml. acetic acid, 0.05 *M*	10	5	2.5	1.25
ml. distilled water	6	11	13.5	14.75

To each solution (20 ml.) are added 1 ml. of a 1 per cent solution of purified egg albumin, or of ash-free gelatin, and 2 drops of a suspension of collodion particles* in distilled water. The protein forms an invisible film on the particles, as is inferred from their behavior in an electrical field. This behavior may be observed in a small cell having a flat observation chamber connected to two vertical side tubes with a bulb at the base of each

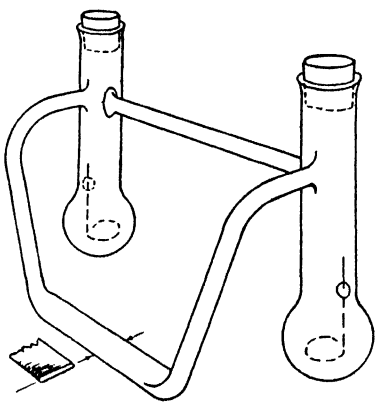

FIG. 28. Cataphoresis cell.†

(Fig. 28). The side tubes are connected by a solid rod to strengthen the apparatus. Through each bulb is sealed a short piece of platinum wire, to which has been fused a piece of silver

* Suitable particles may be obtained by dissolving nitrocellulose in acetone, adding about an equal volume of water, discarding the precipitated mass, and finally removing the acetone by prolonged aeration. The experiment may also be done with quartz particles according to Abramson (1).

† Cells of this type were designed and constructed by Dr. L. F. Nims, and will be described by him elsewhere.

wire. The silver wires, which are coated electrolytically with silver chloride, are the electrodes. Each bulb is filled to a level above the electrode with 0.2 *M* sodium chloride solution, which may be colored with methylene blue to make the boundary more easily visible. The suspension is then carefully added from a pipette so that it floats above the salt solution, with boundaries as sharp as possible, and fills completely the middle and upper parts of the cell. Great care should be taken to keep the salt solution from mixing with the suspension, as the experiment is likely to fail if the suspension in the central part of the cell is contaminated by much electrolyte. When the cell has been completely filled it is closed by rubber stoppers which are carefully introduced without enclosing any air. The cell is then fixed on the stage of a microscope and observed with low power magnification (10 × objective and 10 × ocular). The particles are best seen with dark ground illumination, which may be obtained by removing the upper element of the condenser and putting a black disk below the center of the condenser. It should be possible to focus on the inner surface of either the upper or lower wall of the cell. By noting the distance between these walls in terms of the scale divisions of the fine adjustment, the latter is set for focusing on objects at exactly half the depth of the cell. (If particles are not visible at this depth, help should be obtained from an instructor.) A micrometer disk is put in the ocular of the microscope. The measurement is made by recording with a stop watch the time required for a single particle to move a definite number of divisions (preferably 10) on the ocular micrometer scale. Particular note should be made of the direction of the motion with reference to the electrical polarity of the cell. The apparent direction of motion, as seen through the microscope, is the reverse of the real direction. The electrical field is obtained by connecting the electrodes to a reversing switch which is connected, *with a lamp in series*, to the 110 volt direct current line.* Owing to the high resistance of the cell contents, the potential drop in the suspension is practically 110 volts. The switch should be closed only for the few seconds required to make an observation. The polarity of the house cur-

* Or to a radio battery of 135 volts.

rent may be determined by applying the leads, with the lamp still in series, to a piece of filter paper wet with a dilute solution of sodium chloride containing phenolphthalein. A red color appears at the negative pole.

In measuring the velocities of migration, care should be taken to observe particles in the same part of the cell, as nearly as possible in the center of the flat portion. Several readings should be taken with the particles moving in each direction; equal and opposite velocities should be obtained on reversing the direction of the current. It will be well to use the more acid and more alkaline buffers first; if the particles in these move in opposite directions, then the isoelectric point may be located more exactly by the use of the intermediate buffers.

The observed velocities, in scale divisions per second, are plotted against the pH values of the buffer solutions. In exact work, these pH values are measured with the hydrogen electrode. Velocities should be recorded as positive when the particles move towards the negative electrode, and *vice versa*. A smooth curve is drawn as close as possible to all of the observed points. The isoelectric point of the material under investigation should be given by the pH value at which the curve crosses the line of zero velocity.

The migration of microscopic particles in an electric field was observed by Quincke in 1859. The reversal of the sign of the charge on protein particles with changes in acidity was discovered by Hardy in 1899. The isoelectric points of several proteins were determined by Michaelis by a larger scale method based on the migration of protein in a U-tube. The cataphoresis of collodion particles coated with protein was studied by Loeb (76) using the microscopic method with a flat cell designed by Northrop (94). Similar cells have been used by Abramson (1) in studies of the cataphoresis of red blood cells and of quartz particles coated with protein.

REFERENCES

76. LOEB, Chapter XVII.
82. MICHAELIS, Experiments 52, 53.
98. PAULI and VALKÓ, Chapters V and X.

EXPERIMENT 11

Hydrogen Ions and Enzyme Action

The object of the experiment is to study the effect of pH on the velocity of a typical enzyme action. The reaction to be studied is the transformation of urea into ammonium carbonate in the presence of the enzyme urease. The extent of the reaction is determined by absorbing in standard hydrochloric acid the ammonia set free from the ammonium carbonate formed.

The hydrogen ion concentration is to be varied by using the following phosphate solutions, which are prepared in test tubes 200 × 25 mm. in size.

Approximate pH	5.3	6.2	6.8	7.7	9.2
ml. sodium di-hydrogen phosphate, 0.2 *M*	9.75	8	5	1	0
ml. di-sodium hydrogen phosphate, 0.2 *M*	0.25	2	5	9	10

To each of these phosphate solutions are added 10 ml. of a 2 per cent solution of urea, and one drop of caprylic alcohol to prevent foaming. Each of the large test tubes is provided with a rubber stopper carrying an inlet tube which extends to the bottom and an outlet tube which extends only just through the stopper. The outlet tube is shaped like the middle part of a volumetric pipette, so that the bulb may act as a trap for spattered drops. Each outlet tube is connected by rubber tubing to the inlet tube of a similar large test tube which contains exactly 25 ml. of standard 0.02 *M* hydrochloric acid, a few drops of methyl red indicator solution, and a drop of caprylic alcohol. This tube has also an outlet with a trap. The pairs of tubes are connected in series by rubber tubing so that a single current of air may be drawn through the 10 tubes, the order being such that in each pair of tubes the air passes first through one of the reaction mixtures and then through a tube of standard acid. The current of air is produced by connecting the last tube in the series to the vacuum line. The air entering the first tube from the laboratory should be freed from possible traces of ammonia by passage through an additional tube or wash bottle containing dilute sulphuric acid. Before starting the flow of air the reaction must be started by the addition of 1 ml. of a solution of the enzyme

urease to each of the 5 urea solutions. The exact time when the enzyme is added should be noted in each case. The urease solution is prepared by dissolving 50 mg. of a dry enzyme preparation* in 10 ml. of water. Since the enzyme may not be completely soluble, the mixture should be stirred while each sample is being taken. The reaction in each tube is allowed to proceed for exactly 30 minutes while a gentle current of air is drawn through the whole system. At the end of the alloted time, the air current is shut off and the reaction is stopped by removing the stopper and quickly adding to each urea solution 2 ml. of concentrated alkali (50 per cent sodium hydroxide). The stoppers are replaced at once, and the ammonia liberated is driven over into the standard acid by aeration, using a slow air current for 2 minutes and then as fast a current as is possible without spattering alkali into the acid. After half an hour of rapid aeration, the tubes are disconnected, and the amount of ammonia which has been driven into each acid solution is determined by titrating the excess acid with standard sodium hydroxide solution, 0.02 *M*.

From the titration data the velocity of the reaction in each solution is obtained as ml. of 0.02 *M* ammonia formed per minute of the reaction period. A curve is plotted showing the reaction velocity as a function of the pH of the reaction mixture. This may be assumed to be equal to the pH of the original buffer mixture, although in precise work it would be measured electrometrically in a duplicate solution without the addition of the concentrated alkali.

Students should explain the need for a buffer mixture in studying this reaction. Explanation should also be given of the assumptions used in taking the average velocity as a measure of the true reaction velocity, with discussion of the probable course of the reaction if it were allowed to approach completion. The percentage completion actually reached may be calculated

* Urease preparations are obtainable through the usual laboratory supply houses. Each preparation should be tested, and the amount of enzyme used in this experiment should produce, in 30 minutes at pH 6.8, an amount of ammonia equivalent to about 20 ml. of 0.02 *M* hydrochloric acid.

from the ratio of the amount of ammonia formed to that theoretically obtainable if all of the urea were decomposed.

The effect of the hydrogen ion concentration on the velocity of enzyme reactions became generally known as a result of the careful work of Sørensen (110) who studied the enzymes invertase, catalase, and pepsin. Urease was studied by Van Slyke and Zacharias (121). The occurrence of an optimum pH, or optimum zone of acidity, has been found by numerous subsequent workers to be characteristic of enzyme reactions in general.

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EXPERIMENT 12

REACTION VELOCITY

The reaction of sucrose with water to form glucose and fructose has usually been studied by using a polarimeter to measure changes in optical rotation. This reaction is also accompanied by a small but readily detectable decrease in volume, and the rate of the reaction may be followed by observing the height of a meniscus of the solution in a capillary tube connected to a closed vessel kept at constant temperature. Such a vessel is called a dilatometer.

The reaction proceeds very slowly at ordinary temperatures unless a suitable catalyst is present. Such a catalyst is an acid which provides a high concentration of hydrogen ions, or the enzyme sucrase (or invertase) which may be obtained from yeast or from animal intestines. It is instructive for neighboring students to perform the experiment simultaneously with hydrochloric acid and with yeast invertase as catalyst. In either case two workers are needed to start the experiment.

Catalysis by Acid. Twenty grams of sugar are dissolved in 50 ml. of distilled water in an Erlenmeyer flask. Fifty ml. of 2.0 *M* hydrochloric acid are placed in another flask. The flasks are

supported in a constant temperature bath accurately regulated at 30°C.

The dilatometer consists of a glass cylinder holding about 30 ml. with a stop-cock fused on its lower end and a capillary tube about 50 cm. long and 0.6 mm. in diameter fused on its upper end. The dilatometer may be cleaned by forcing distilled water through it by means of a suction pump, and dried by a current of air. The capillary tube should be provided with a scale of millimeter paper with the centimeter marks numbered upwards. Time may be recorded with an ordinary watch set so that the second hand passes 60 when the minute hand crosses a minute line. After the solutions have been in the bath about 15 minutes, the experiment may be started by removing the flasks from the bath, wiping them, and mixing the two solutions as rapidly as possible by pouring them into a single flask and shaking. The mixing should be done as nearly as possible when the second hand of the watch crosses 60, and the time in minutes should be recorded at once. The dilatometer is filled from below by pressure, the solution being forced by compressed air up from the flask through a vertical tube connected by a short piece of rubber tubing to the lower end of the dilatometer. It may be necessary to hold the stopper in the flask by hand during the filling. The filling should take place slowly enough to avoid getting air bubbles in the dilatometer. Great care is necessary during the filling of the upper part of the tube and the capillary. The stop-cock should be nearly closed when the level is a few millimeters below the bottom of the capillary, and tightly closed as soon as the liquid has gone up to within a few centimeters of the top of the scale. The dilatometer is disconnected from the flask and clamped in the bath in a vertical position, with the wide part entirely submerged. Readings are begun at once, the height being recorded to the nearest hundredth of a centimeter, as estimated by looking through a magnifying glass. At first readings should be taken every minute and later the time intervals should be extended to correspond to about 1 cm. fall in level. It should be possible to get the first reading not more than 5 minutes after the reaction was started, and readings during the

next 5 or 10 minutes may be somewhat irregular, due to temperature changes. The regularity of the course of the reaction is tested by plotting the readings in centimeters against the time in minutes from the start of the reaction. Readings should be continued at intervals of about 1 cm. for about 2 hours, and the apparatus should be left undisturbed for several hours until a final minimum reading is obtained. After about 8 hours the volume begins to increase, presumably because of some secondary reaction.

The logarithm of the difference between the reading at each time and the final reading is plotted against the reaction time. The value of the unimolecular velocity constant is obtained from the curve, and the curve is extrapolated to get a reading corresponding to zero time. This extrapolated value is plotted on the original curve of readings against time, and its value compared with that obtained by drawing the best smooth curve through or near the observed points.

Catalysis by an Enzyme. Twenty grams of cane sugar are dissolved in 85 ml. of water, and 10 ml. of an acetate buffer solution of pH 4.6, 0.1 *M* in acetic acid and in sodium acetate, are added. In a separate flask are put 5 ml. of a solution of the enzyme invertase, previously prepared from yeast.* The solutions are brought to the temperature of the bath, and the reaction is started, the dilatometer filled, and the observations taken, exactly as in the preceding section. It should be noted that no strong acid is used, because this enzyme exhibits its maximum activity only between pH 4 and 5. The final volume in this experiment with invertase usually remains constant for several days.

The results are plotted as in the preceding section, and any differences in the form of the curves from those obtained in the experiment with the acid catalyst should be noted. A decision may be reached as to whether this reaction follows the uni-

* Invertase may be prepared from yeast in the laboratory, or purchased from certain commercial laboratories (8, p. 370). The concentration of the enzyme should be adjusted to make the reaction about 90 per cent complete in 2 hours.

molecular equation by inspecting the semilogarithmic plot From the nature of this curve it should be possible to decide whether the unimolecular velocity coefficient is constant, or whether it increases or decreases with time. The decision should be confirmed by calculating the values of this coefficient from three widely separated experimental points.

The kinetics of the acid hydrolysis of cane sugar was studied by Wilhelmy in 1850. In the case of invertase hydrolysis, divergences from the unimolecular equation have been found by most workers, including Michaelis (86) and Nelson (88, 90). The explanation of the kinetics of invertase action is still a matter of argument. Dilatometers have recently been used Sreenivasaya (112) and by Rona (102) in studying enzyme reactions.

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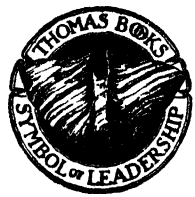

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