greater reduction of surface tension that occurs at lower concentrations for longer chain-length compounds. In addition, note the greater slopes with increasing concentration, indicating more adsorption (Eq 30), and the abrupt leveling of surface tension at higher concentrations. This latter behavior reflects the self-association of surface-active agent to form micelles which exhibit no further tendency to reduce surface tension. The topic of micelles will be discussed later on page 268.

If one plots the values of surface concentration, Γ, vs concentration, c, for substances adsorbing to the vapor-liquid and liquid-liquid interfaces, using data such as those given in Fig 19-13, one generally obtains an adsorption isotherm shaped like those in Fig 19-9 for vapor adsorption. Indeed, it can be shown that the Langmuir equation (Eq 25) can be fitted to such data when written in the form

$$\Gamma = \frac{\Gamma_{max} k'c}{1 + k'c} \quad (31)$$

where Γ_{max} is the maximum surface concentration attained with increasing concentration and k' is related to k in Eq 25. Combining Eqs 29 and 31 leads to a widely used relationship between surface tension change Π (see Eq 28) and solute concentration, c, known as the Syszkowski equation:

$$\Pi = \Gamma_{max} RT \ln (1 + k'c) \quad (32)$$

Mixed Films

It would seem reasonable to expect that the properties of a surface film could be varied greatly if a mixture of surface-active agents were in the film. As an example, consider that a mixture of short- and long-chain fatty acids would be expected to show a degree of "condensation" varying from the "gaseous" state, when the short-chain substance is used in high amount, to a highly condensed state when the longer chain substance predominates. Thus, each component in such a case would operate independently by bringing a proportional amount of film behavior to the system.

More often, the ingredients of a surface film do not behave independently, but, rather, interact to produce a new surface film. An obvious example would be the combination of organic amines and acids which are oppositely charged and would be expected to interact strongly.

In addition to such polar-group interactions, chain–chain interaction will strongly favor mixed condensed films. An important example of such a case occurs when a long-chain alcohol is introduced along with an ionized long-chain substance. Together the molecules form a highly condensed film despite the presence of a high number of like charges. Presumably this occurs as seen in Fig 19-14, by arranging the molecules so that ionic groups alternate with alcohol groups; however, if chain–chain interactions are not strong, the ionic species often will be displaced by the more nonpolar unionized species and "desorb" into the bulk solution.

On the other hand, sometimes the more soluble surface-active agent produces surface pressures in excess of the collapse pressure of the insoluble film and displaces it from the surface. This is an important concept because it is the underlying principle behind cell lysis by surface-active agents and some drugs, and behind the important process of detergency.

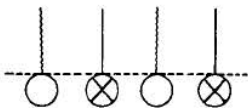

Fig 19-14. A mixed monomolecular film. ⊗: a long-chain ion; O: a long-chain nonionic compound.

Adsorption on Solid Surfaces From Solution

Adsorption to solid surfaces from solution may occur if dissolved molecules and the solid surface have chem groups capable of interacting. Nonspecific adsorption will occur if the solute is surface active and if the surface of the solid is high. This latter case would be the sam occurs at the vapor-liquid and liquid-liquid interfaces. with adsorption to liquid interfaces, adsorption to solid faces from solution generally leads to a monomolecular er, often described by the Langmuir equation or by empirical, yet related, Freundlich equation

$$x/M = kc^n$$

where x is the grams of solute adsorbed by M grams of s in equilibrium with a solute concentration of c. The terr and n are empirical constants. However, as Giles[8] pointed out, the variety of combinations of solutes and ids, and, hence the variety of possible mechanisms of ads tion, can lead to a number of more complex isotherms. particular, adsorption of surfactants and polymers, of g importance in a number of pharmaceutical systems, is not well understood on a fundamental level, and ma some situations even be multilayered.

Adsorption from solution may be measured by separa solid and solution and either estimating the amount of sorbate adhering to the solid or the loss in concentratio adsorbate from solution.

In view of the possibility of solvent adsorption, the la approach really only gives an apparent adsorption. example, if solvent adsorption is great enough, it is poss to end up with an increased concentration of solute a contact with the solid; here, the term negative adsorptic used.

Solvent not only influences adsorption by competing the surface but, as discussed in connection with adsorp at liquid surfaces, the solvent will determine the escap tendency of a solute; eg, the more polar the molecule, the the adsorption that occurs from water. This is seen in 19-15, where adsorption of various fatty acids from w onto charcoal increases with increasing alkyl chain lengt nonpolarity. It is difficult to predict these effects but general, the more chemically unlike the solute and solv and the more alike the solid surface groups and solute,

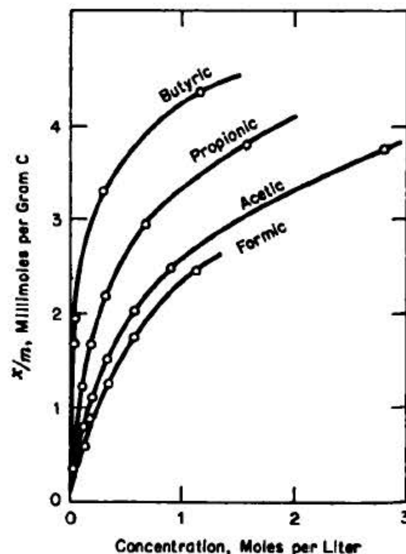

Fig 19-15. The relation between adsorption and molecular weig fatty acids.[9]

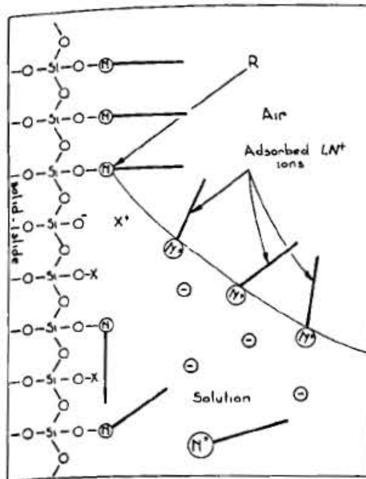

Fig 19-16. The adsorption of a cationic surfactant, LN^+, onto a negatively charged silica or glass surface, exposing a hydrophobic surface as the solid is exposed to air.[10]

greater the extent of adsorption. Another factor which must be kept in mind is that charged solid surfaces, such as polyelectrolytes, will strongly adsorb oppositely charged solutes. This is similar to the strong specific binding seen in gas chemisorption and it is characterized by significant monolayer adsorption at very low concentrations of solute. See Fig 19-16 for an example of such adsorption.

Surface-Active Agents

Throughout the discussion so far, examples of surface-active agents (surfactants) have been restricted primarily to fatty acids and their salts. It has been shown that both a hydrophobic portion (alkyl chain) and a hydrophilic portion (carboxyl and carboxylate groups) are required for their surface activity, the relative degree of polarity determining the tendency to accumulate at interfaces. It now becomes important to look at some of the specific types of surfactants available and to see what structural features are required for different pharmaceutical applications.

The classification of surfactants is quite arbitrary, but one based on chemical structure appears best as a means of introducing the topic. It is generally convenient to categorize surfactants according to their polar portions since the nonpolar portion is usually made up of alkyl or aryl groups. The major polar groups found in most surfactants may be divided as follows: anionic, cationic, amphoteric and nonionic. As we shall see, the last group is the largest and most widely used for pharmaceutical systems, so that it will be emphasized in the discussion that follows.

Types

Anionic Agents—The most commonly used anionic surfactants are those containing carboxylate, sulfonate, and sulfate ions. Those containing carboxylate ions are known as soaps and are generally prepared by the saponification of natural fatty acid glycerides in alkaline solution. The most common cations associated with soaps are sodium, potassium, ammonium, and triethanolamine, while the chain length of the fatty acids ranges from 12 to 18.

The degree of water solubility is greatly influenced by the length of the alkyl chain and the presence of double bonds. For example, sodium stearate is quite insoluble in water at room temperature, whereas sodium oleate under the same conditions is quite water soluble.

Table VII—Effect of Aerosol OT Concentration on the Surface Tension of Water and the Contact Angle of Water with Magnesium Stearate

Concentration, $m \times 10^6$	γ_{sv}	θ
1.0	60.1	120°
3.0	49.8	113°
5.0	45.1	104°
8.0	40.6	89°
10.0	38.6	80°
12.0	37.9	71°
15.0	35.0	63°
20.0	32.4	54°
25.0	29.5	50°

Multivalent ions, such as calcium and magnesium, produce marked water insolubility, even at lower alkyl chain lengths; thus, soaps are not useful in hard water which is high in content of these ions. Soaps, being salts of weak acids, are subject also to hydrolysis and the formation of free acid plus hydroxide ion, particularly when in more concentrated solution.

To offset some of the disadvantages of soaps, a number of long-alkyl-chain sulfonates, as well as alkyl aryl sulfonates such as sodium dodecylbenzene sulfonate, may be used; the sulfonate ion is less subject to hydrolysis and precipitation in the presence of multivalent ions. A popular group of sulfonates, widely used in pharmaceutical systems, are the dialkyl sodium sulfosuccinates, particularly sodium bis-(2-ethylhexyl)sulfosuccinate, best known as Aerosol OT or docusate sodium. This compound is unique in that it is both oil and water soluble and hence forms micelles in both phases. It reduces surface and interfacial tension to low values and acts as an excellent wetting agent in many types of solid dosage forms (see Table VII).

A number of alkyl sulfates are available as surfactants, but by far the most popular member of this group is sodium lauryl sulfate, which is widely used as an emulsifier and solubilizer in pharmaceutical systems. Unlike the sulfonates, sulfates are susceptible to hydrolysis which leads to the formation of the long-chain alcohol, so that pH control is most important for sulfate solutions.

Cationic Agents—A number of long-chain cations, such as amine salts and quaternary ammonium salts, are often used as surface-active agents when dissolved in water; however, their use in pharmaceutical preparations is limited to that of antimicrobial preservation rather than as surfactants. This arises because the cations adsorb so readily at cell membrane structures in a nonspecific manner, leading to cell lysis (eg, hemolysis), as do anionics to a lesser extent. It is in this way that they act to destroy bacteria and fungi.

Since anionic and nonionic agents are not as effective as preservatives, one must conclude that the positive charge of these compounds is important; however, the extent of surface activity has been shown to determine the amount of material needed for a given amount of preservation. Quaternary ammonium salts are preferable to free amine salts since they are not subject to effect by pH in any way; however, the presence of organic anions such as dyes and natural polyelectrolytes is an important source of incompatibility and such a combination should be avoided.

Amphoteric Agents—The major group of molecules falling into this category are those containing carboxylate or phosphate groups as the anion and amino or quaternary ammonium groups as the cation. The former group is represented by various polypeptides, proteins, and the alkyl betaines, while the latter group consist of natural phospholipids such as the lecithins and cephalins. In general, long-chain amphoterics which exist in solution in zwitterionic form are

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more surface-active than ionic surfactants having the same hydrophobic group since in effect the oppositely charged ions are neutralized. However, when compared to nonionics, they appear somewhere between ionic and nonionic.

Nonionic Agents—The major class of compounds used in pharmaceutical systems are the nonionic surfactants since their advantages with respect to compatibility, stability, and potential toxicity are quite significant. It is convenient to divide these compounds into those that are relatively water insoluble and those that are quite water soluble.

The major type of compounds making up this first group are the long-chain fatty acids and their water-insoluble derivatives. These include (1) fatty alcohols such as lauryl, cetyl (16 carbons) and stearyl alcohols; (2) glyceryl esters such as the naturally occurring mono-, di- and triglycerides; and (3) fatty acid esters of fatty alcohols and other alcohols such as propylene glycol, polyethylene glycol, sorbitan, sucrose and cholesterol. Included also in this general class of nonionic water-insoluble compounds are the free steroidal alcohols such as cholesterol.

To increase the water solubility of these compounds and to form the second group of nonionic agents, polyoxyethylene groups are added through an ether linkage with one of their alcohol groups. The list of derivatives available is much too long to cover completely, but a few general categories will be given.

The most widely used compounds are the polyoxyethylene sorbitan fatty acid esters which are found in both internal and external pharmaceutical formulations. Closely related compounds include polyoxyethylene glyceryl, and steroidal esters, as well as the comparable polyoxypropylene esters. It is also possible to have a direct ether linkage with the hydrophobic group as with a polyoxyethylene–stearyl ether or a polyoxyethylene–alkyl phenol. These ethers offer advantages since, unlike the esters, they are quite resistant to acidic or alkaline hydrolysis.

Besides the classification of surfactants according to their polar portion, it is useful to have a method that categorizes them in a manner that reflects their interfacial activity and their ability to function as wetting agents, emulsifiers, solubilizers, etc. Since variation in the relative polarity or nonpolarity of a surfactant significantly influences its interfacial behavior, some measure of polarity or nonpolarity should be useful as a means of classification.

One such approach assigns a hydrophile–lipophile balance number (HLB) for each surfactant and, although developed by a commercial supplier of one group of surfactants, the method has received wide-spread application. The HLB value, as originally conceived for nonionic surfactants, is merely the percentage weight of the hydrophilic group divided by five in order to reduce the range of values. On a molar basis, therefore, a 100% hydrophilic molecule (polyethylene glycol) would have a value of 20.

Thus, an increase in polyoxyethylene chain length increases polarity and, hence, the HLB value; at constant polar chain length, an increase in alkyl chain length or number of fatty acid groups decreases polarity and the HLB value. One immediate advantage of this system is that to a first approximation one can compare any chemical type of surfactant to another type when both polar and nonpolar groups are different.

HLB values for nonionics are calculable on the basis of the proportion of polyoxyethylene chain present; however, in order to determine values for other types of surfactants it is necessary to compare physical chemical properties reflecting polarity with those surfactants having known HLB values.

Relationships between HLB and phenomena such as water solubility, interfacial tension, and dielectric constant have been used in this regard. Those surfactants exhibiting values greater than 20 (eg, sodium lauryl sulfate) demonstrate hydrophilic behavior in excess of the polyoxyethylene groups alone. Table XIX, page 304, presents HLB values for a variety of surface-active agents.

Surfactant Properties in Solution and Micelle Formation

As seen in Fig 19-13, increasing the concentration of surface-active agents in aqueous solution causes a decrease in the surface tension of the solution until a certain concentration where it then becomes essentially constant with increasing concentration. That this change is associated with changes also taking place in the bulk solution rather than just at the surface can be seen in Fig 19-17, which shows the same abrupt change in bulk solution properties such as solubility, equivalent conductance and osmotic pressure as with surface properties. The most reasonable explanation for these effects is that the solute molecules self-associate to form soluble aggregates which exhibit markedly different properties from the monomers in solution. Such aggregates (Fig 19-18*A*) appear to exhibit no tendency to adsorb to the surface since the surface and interfacial tension above this solute concentration do not change to any significant extent. Such aggregates, known as micelles, form over such a very narrow range of concentrations that one can speak of a critical micellization concentration (cmc). These micelles form for essentially the same reasons that cause molecules to be adsorbed; the lack of affinity of the hydrophobic chains for water molecules and the tendency for strong hydrophobic chain-chain interactions when the chains are oriented closely together in the micelle, coupled with the gain in entropy due to the loss of the ice-like structure of water when the chains are separated from water, lead to a favorable free energy change for micellization. The longer the hydrophobic chain or the less the polarity of the polar group, the greater the tendency for monomers to "escape" from the water to form micelles and, hence the lower the cmc (see Fig 19-13).

In dilute solution (still above the cmc) the micelles can be considered to be approximately spherical in shape (Fig 19-18*A* and *B*), while at higher concentrations they become

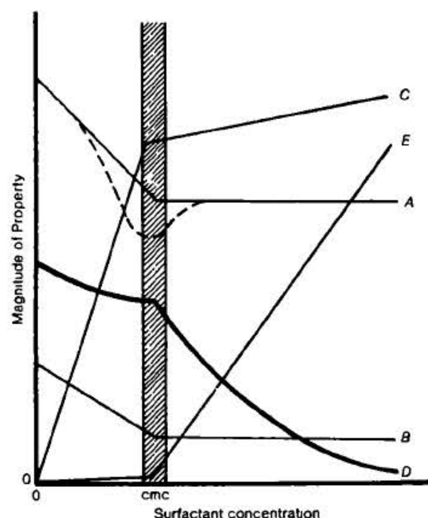

Fig 19-17. Effect of surfactant concentration and micelle formation on various properties of the aqueous solution of an ionic surfactant. *A:* Surface tension; *B:* interfacial tension; *C:* osmotic pressure; *D:* equivalent conductivity; *E:* solubility of compound with very low solubility in pure water [11]

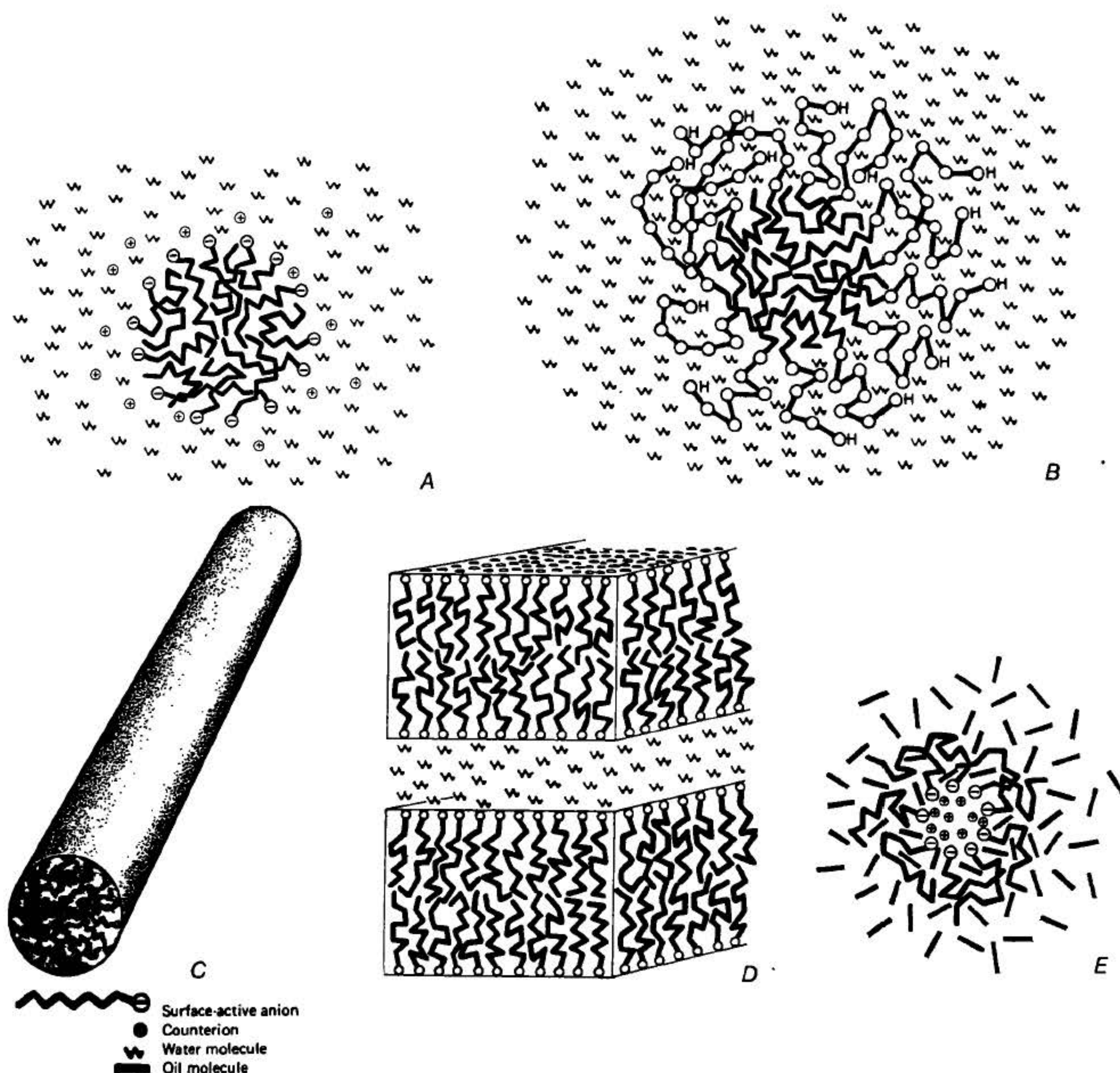

Fig 19-18. Different types of micelles. *A:* Spherical micelle of an anionic surfactant; *B:* spherical micelle of a nonionic surfactant; *C:* cylindrical micelle of an ionic surfactant; *D:* lamellar micelle of an ionic surfactant; *E:* reverse micelle of an anionic surfactant in oil.[11]

more asymmetric and eventually assume cylindrical (Fig 19-18*C*) or lamellar (Fig 19-18*D*) structures. It is important to recognize that equilibrium, and hence reversibility, exists between the monomers and the various types of micelles. The sizes of such micelles depend on the number of monomers per micelle and the size and molecular shape of the individual monomers. In Table VIII are given the cmc and number of monomers per micelle for different types of surfactants. Note for the nonionic surfactants that the longer the polyoxyethylene chain, and hence the more polar and bulkier the molecule, the higher the cmc, ie the less the tendency for micelle formation. It is also possible for oil-soluble surfactants to show a tendency to self-associate into "reverse micelles in nonpolar solvents, as depicted in Fig 19-18*E*, with their polar groups all oriented away from the solvent. In general these micelles tended to be smaller and to aggregate over a wider range of concentrations than seen in water, and therefore, to exhibit no well-defined cmc.

Micellar Solubilization

As seen in Fig 19-18, the interior of surfactant micelles formed in aqueous media consists of hydrocarbon "tails" in liquid-like disorder. The micelles, therefore, resemble miniscule pools of liquid hydrocarbon surrounded by shells of polar "head groups." Compounds which are poorly soluble in water but soluble in hydrocarbon solvents, can be dissolved inside these micelles, ie, they are brought homogeneously into an overall aqueous medium.

Being hydrophobic and oleophilic, the solubilized molecules are located primarily in the hydrocarbon core of the micelles (see Fig 19-19*A*). Even water-insoluble drugs usually contain polar functional groups such as hydroxyl, carbonyl, ether, amino, amide, and cyano. Upon solubilization, these hydrophilic groups locate on the periphery of the micelle among the polar headgroups of the surfactant in order to become hydrated (see Fig 19-19*B*). For instance,

Table VIII—Critical Micelle Concentrations and Micellar Aggregation Numbers of Various Surfactants in Water at Room Temperature

Structure	Name	CMC, mM/L	Surfactant molecules/ micelle
n-$C_{11}H_{23}COOK$	Potassium laurate	24	50
n-$C_8H_{17}SO_3Na$	Sodium octant sulfonate	150	28
n-$C_{10}H_{21}SO_3Na$	Sodium decane sulfonate	40	40
n-$C_{12}H_{25}SO_3Na$	Sodium dodecane sulfonate	9	54
n-$C_{12}H_{25}OSO_3Na$	Sodium lauryl sulfate	8	62
n-$C_{12}H_{25}OSO_3Na$	Sodium lauryl sulfate[a]	1	96
	Sodium di-2-ethylhexyl sulfosuccinate	5	48
n-$C_{10}H_{21}N(CH_3)_3Br$	Decyltrimethylammonium bromide	63	36
n-$C_{12}H_{25}N(CH_3)_3Br$	Dodecyltrimethylammonium bromide	14	50
n-$C_{14}H_{29}N(CH_3)_3Br$	Tetradecyltrimethylammonium bromide	3	75
n-$C_{14}H_{29}N(CH_3)_3Cl$	Tetradecyltrimethylammonium chloride	3	64
n-$C_{12}H_{25}NH_3Cl$	Dodecylammonium chloride	13	55
n-$C_{12}H_{25}O(CH_2CH_2O)_8H$	Octaoxyethylene glycol monododecyl ether	0.13	132
n-$C_{12}H_{25}O(CH_2CH_2O)_8H$[b]		0.10	301
n-$C_{12}H_{25}(CH_2CH_2O)_{12}H$	Dodecaoxyethylene glycol monododecyl ether	0.14	78
n-$C_{12}H_{25}O(CH_2CH_2O)_{12}H$[b]		0.091	116
t-C_8H_{17}-C_6H_4-$O(CH_2CH_2O)_{9.7}H$	Decaoxyethylene glycol mono-p,t-octylphenyl ether (octoxynol 9)	0.27	100

[a] Interpolated for physiologic saline, 0 154 M NaCl.
[b] At 55° instead of 20°.

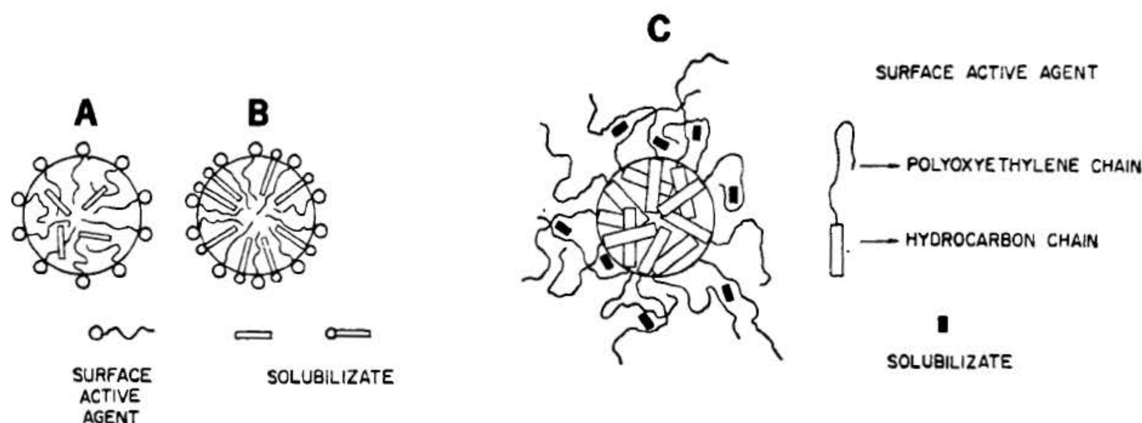

Fig 19-19. The locations of solubilizates in spherical micelles. A: Ionic surfactant (solubilized molecule has no hydrophilic groups); B: Ionic surfactant (solubilized molecule has a hydrophilic group); C: nonionic surfactant (polar solubilizate) [12]

when cholesterol or dodecanol is solubilized by sodium lauryl sulfate, their hydroxyl groups penetrate between sulfate ions and are even bound to them by hydrogen bonds, while their hydrocarbon portions are immersed among the dodecyl tails of the surfactant which make up the core of the micelle.

Micelles of polyoxyethylated nonionic surfactants consist of an outer shell of hydrated polyethylene glycol moieties and a core of hydrocarbon moieties. Compounds like phenol, cresol, benzoic acid, salicylic acid, and esters of p-hydroxy and p-aminobenzoic acids have some solubility in water and in oils but considerable solubility in liquids of intermediate polarity like ethanol, propylene glycol or aqueous solutions of polyethylene glycols. When solubilized by nonionic micelles, they are located in the hydrated outer polyethylene glycol shell as shown in Fig 19-19C. Since these compounds have hydroxyl or amino groups, they frequently form complexes with the ether oxygens of the surfactant by hydrogen bonding.

Solubilization is generally nonspecific: any drug which is appreciably soluble in oils can be solubilized. Each has a solubilization limit, comparable to a limit of solubility, which depends on temperature and on the nature and concentration of the surfactant. Hartley distinguishes two categories of solubilizates. The first consists of comparatively large, asymmetrical and rigid molecules forming crystalline solids, such as steroids and dyes. These do not blend in with the normal paraffin tails which make up the micellar core; because of dissimilarity in structure, they remain distinct as solute molecules. They are sparingly solubilized by surfactant solutions, a few molecules/micelle at saturation (see Table IX). The number of carbon atoms in the micellar hydrocarbon core required to solubilize a molecule of steroid or dye at saturation is of the same order of magnitude as the number of carbon atoms of bulk liquid dodecane or hexadecane per molecule of steroid or dye in their saturated solutions in these liquids.

Since solubilization depends on the presence of micelles, it does not take place below the cmc. It can, therefore, be used to determine the cmc, particularly when the solubilizate is a dye or another compound easy to assay. Plotting the maximum amount of a water-insoluble dye solubilized by aqueous surfactant, or the absorbance of its saturated solutions, versus the surfactant concentration produces a straight line which intersects the surfactant concentration axis at the cmc. Above the cmc, the amount of solubilized dye is directly proportional to the number of micelles and, therefore,

Table IX—Micellar Solubilization Capacities of Different Surfactants for Estrone[13]

Surfactant	Concentration range, molarity	Temp, °C	Moles surfactant/ mole solubilized estrone
Sodium laurate	0.025–0.023	40	91
Sodium oleate	0.002–0.35	40	53
Sodium lauryl sulfate	0.004–0.15	40	71
Sodium cholate	0.09–0.23	20	238
Sodium deoxycholate	0.007–0.36	20	476
Diamyl sodium sulfosuccinate	0.08–0.4	40	833
Dioctyl sodium sulfosuccinate	0.002–0.05	40	196
Tetradecyltrimethylammonium bromide	0.005–0.08	20	45
Hexadecylpyridinium chloride	0.001–0.1	20	32
Polysorbate 20	0.002–0.15	20	161
Polysorbate 60	0.0008–0.11	20	83

proportional to the overall surfactant concentration. Below the cmc, no solubilization takes place. This is represented by Curve E of Fig 19-17.

The second category of compounds to be solubilized are often liquid at room temperature and consist of relatively small, symmetrical, and/or flexible molecules such as many constituents of essential oils. These molecules mix and blend in freely with the hydrocarbon portions of the surfactants in the core of the micelles, so as to become indistinguishable from them. Such compounds are extensively solubilized and in the process usually swell the micelles: they augment the volume of the hydrocarbon core and increase the number of surfactant molecules per micelle. Their solubilization frequently lowers the cmc.

Microemulsions[14-16]

Microemulsions are liquid dispersions of water and oil that are made homogeneous, transparent, and stable by the addition of relatively large amounts of a surfactant and a cosurfactant. *Oil* is defined as a liquid of low polarity and low miscibility with water, eg, toluene, cyclohexane, mineral or vegetable oils.

Microemulsions are intermediate in properties between micelles containing solubilized oils and emulsions. While emulsions are lyophobic and unstable, microemulsions are on the borderline between lyophobic and lyophilic colloids. True microemulsions are thermodynamically stable.[17] Therefore, they are formed spontaneously when oil, water, surfactants, and cosurfactants are mixed together. The unstable emulsions require input of considerable mechanical energy for their preparation, which may be supplied by colloid mills, homogenizers or ultrasonic generators.

Both emulsions and microemulsions may contain high volume fractions of the internal phase. For instance, some O/W systems contain 75% (v/v) of oil dispersed in 25% water, although lower internal phase volume fractions are more common.

At low surfactant concentrations, viz, low multiples of the cmc, micelles are spheres (Fig 19-18*A*, *B* and *E*) or ellipsoids. When an oil is solubilized by micelles in water, it blends into the micellar core formed by the hydrocarbon tails of the surfactant molecules (Fig 19-19) and swells the micelles.

Spherical or ellipsoidal micelles are nearly monodisperse, and their mean diameters are in the range of 25 to 60 Å. Microemulsion droplets also have a narrow droplet size distribution with a mean diameter range of approximately 60 to 1000 Å. Since the droplet diameters are less than ¼ of the wavelength of light (4200 Å for violet and 6600 Å for red light), microemulsions scatter little light and are, therefore, transparent or at least translucent.

Emulsions have very broad droplet size distributions. Only the smallest droplets, with diameters of about 1000 to 2000 Å, are below the resolving power of the light microscope. The upper size limit is 25 or 50 μm (250,000 or 500,000 Å). Because emulsion droplets are comparable in size, or larger than the wavelength of visible light, they scatter it more or less strongly depending on the difference in refractive index between oil and water. Thus, most emulsions are opaque.

The three disperse systems—micellar solutions, microemulsions, and emulsions—can be of the O/W (oil-in-water) or W/O type. Aqueous micellar surfactant solutions can solubilize oils and lipid-soluble drugs in the core formed by their hydrocarbon chains. Likewise, oil-soluble surfactants like sorbitan monooleate and docusate sodium form "reverse micelles" in oils (Fig 19-18*E*) capable of solubilizing water in the polar center. The solubilized oil in the former micelles and the solubilized water in the latter may in turn enhance the micellar solubilization of oil-soluble and water-soluble drugs, respectively.

Oil-soluble drugs have been incorporated into O/W emulsions by dissolving them in the oil phase before emulsification.[18] By the same token, it may be possible to dissolve oil-soluble drugs in a vegetable oil and make an oral or parenteral O/W microemulsion. The advantage of such microemulsion systems over conventional emulsions is their smaller droplet size and superior shelf stability. Aqueous micellar solutions[19] and O/W microemulsions[20] have both been used as aqueous reaction media for oil-soluble compounds.

Emulsions and micellar solutions of oils solubilized in aqueous surfactant solutions consist of three components, oil, water and surfactant. Microemulsions generally require a fourth component, called *cosurfactant*. Commonly used cosurfactants are linear alcohols of medium chain length, which are sparingly miscible with water. Since the cosurfactants as well as the surfactants are surface-active, they promote the generation of extensive interfaces through the spontaneous dispersion of oil in water, or vice-versa, resulting in the formation of microemulsions. The large interfacial area between oil and water permits the extensive formation of a mixed interfacial film consisting of surfactant and cosurfactant. This film is called the "interphase" because it is thicker than the surfactant monolayers formed at oil-water interfaces in emulsions. The interfacial tension at the oil-water interface in microemulsions approaches zero, which also contributes to their spontaneous formation. According to another viewpoint, microemulsions are regarded as micelles extensively swollen by large amounts of solubilized oil.

Typical formulations for an O/W and a W/O microemulsion are shown in Table X. The ratio, g surfactant/g solubilized or emulsified oil or water is in the range of 2 to 20 for micellar solutions and 0.01 to 0.1 for emulsions. Microemulsions have intermediate values: The ratios for the formulations in Table X are near unity. In industrial formulations,

Table X—Microemulsion Formulations

Compound	Function	Content in microemulsions, % O/W	W/O
Sodium lauryl sulfate	Surfactant	13	10
1-Pentanol	Cosurfactant	8	25
Xylene	Oil	8	50
Water		71	15

the ratios are closer to 0.1 to reduce costs. Microemulsions are used in such diverse applications as floor polish and agricultural pesticide formulations and in tertiary petroleum recovery. The use of O/W microemulsions as aqueous vehicles for oil-soluble drugs to be administered by the percutaneous, oral or parenteral route is being investigated.

Colloidal Dispersions

Historical Background of Colloids

The term *colloid*, derived from the Greek word for glue, was applied *ca* 1850 by the British chemist Thomas Graham to polypeptides such as albumin and gelatin, to vegetable gums such as acacia, starch and dextrin, and to inorganic compounds such as gelatinous metal hydroxides and Prussian blue (ferric ferrocyanide). These compounds did not crystallize, and diffused very slowly when dissolved or dispersed in water. They could be separated from ordinary solutes such as salts and sugar, called "crystalloids," as the latter diffused through the fine pores of dialysis membranes made from animal gut which retained the "colloids." "Crystalloids" crystallized readily from solution.[21,22]

Von Weimarn was the first to identify colloidality as a state of subdivision of matter rather than as a category of substances. Many of Graham's "colloids," especially proteins, have been crystallized. Moreover, von Weimarn was able to prepare all "crystalloids" investigated in the colloidal state. Colloidal dispersions by the condensation method resulted from high relative supersaturation, which produced a large number of small nuclei.[21-23,28] For instance, clear, transparent solidified jellies were prepared by cooling aqueous solutions of $CaCl_2$, $Ba(SCN)_2$ and $Al_2(SO_4)_3$, and aqueous-alcoholic solutions of NaCl, KCl, NH_4Cl, KSCN, NaBr and NH_4NO_3 which were nearly saturated at room temperature.[28]

Colloid chemistry became a science in its own right around 1906, when Wolfgang Ostwald wrote the booklet "The World of the Neglected Dimensions." In it, he focused on colloidal systems as a state of matter that has disperse phases intermediate in size between small molecules or ions in solution and large, visible particles in suspension. Ostwald became the first editor of the journal *Kolloid-Zeitschrift* in 1907. The studies of colloidal systems and surface or interfacial phenomena are intimately related. The properties of colloidal dispersions are largely governed by the nature of the surface of their particles. The division of the American Chemical Society specializing in colloidal systems and interfaces is called the "Division of Colloid and Surface Chemistry," while the pertinent session of the Gordon Research Conferences is called "Chemistry at Interfaces."

Colloid and surface chemistry deals with an unusually wide variety of industrial and biological systems. A few examples are catalysts, lubricants, adhesives, latexes for paints, rubbers and plastics, soaps and detergents, clays, packaging films, cigarette smoke, liquid crystals, cell membranes, mucous secretions and aqueous humors.

Definitions and Classifications

Colloidal Systems and Interfaces

Colloidal dispersions consist of at least two discrete phases, namely, one or more disperse, dispersed or internal phases and a continuous or external phase called the *dispersion medium* or *vehicle*. What distinguishes colloidal dispersions from solutions and coarse dispersions is the particle size of the disperse phase. Systems in the colloidal state contain one or more substances that have at least one dimension in the range of 10 to 100 Å (1 Angstrom unit = 10^{-8} cm = 10^{-10} m) or 1–10 nm (1 nanometer = 10^{-9} m) at the lower end, and a few micrometers (μm) at the upper end (1 μm = 10^4 Å = 10^{-6} m). Thus blood, cell membranes, the thinner nerve fibers, milk, rubber latex, fog and beer foam are colloidal systems. Some types of materials, such as many emulsions, and oral suspensions of most organic drugs, are coarser than true colloidal systems but exhibit similar behavior. Even though serum albumin, acacia and povidone form true or molecular solutions in water, the size of the individual solute molecules places such solutions in the colloidal range (particle size > 10 Å).[21-27]

The following features distinguish colloidal dispersions from coarse suspensions. Disperse particles in the colloidal range are usually too fine to be visible in a light microscope, because at least one dimension measures 1 μm or less. They are often visible in the ultramicroscope and always in the electron microscope. Coarse suspended particles are frequently visible to the naked eye and always in the light microscope. Colloidal particles, as opposed to coarse particles, pass through ordinary filter paper but are retained by dialysis or ultrafiltration membranes. Because of their small size, colloidal dispersions undergo little or no sedimentation or creaming: Brownian motion maintains the disperse particles in suspension (see below).

Except for high polymers, most soluble substances can be prepared either as low-molecular-weight solutions, or as colloidal dispersions or coarse suspensions depending on the choice of the dispersion medium and the dispersion technique.[26,28]

Because of the small size of colloidal particles, appreciable fractions of their atoms, ions or molecules are located in the boundary layer between a particle and air (surface) or between a particle and a liquid or solid (interface). The ions in the surface of a sodium chloride crystal and the water molecules in the surface of a rain drop are subjected to unbalanced forces of attraction, whereas the ions or molecules in the interior of the materials are surrounded by similar ions or molecules on all sides, with balanced force fields. Thus a surface free energy component is added to the total free energy of colloidal particles, which becomes relatively more important as the particles become smaller, ie, as greater fractions of their ions, atoms or molecules are located in their surface or interfacial region. Hence the solubility of very fine solid particles and the vapor pressure of very small liquid droplets are larger than the corresponding values of coarse particles and large drops of the same materials, respectively.

Specific Surface Area—Decreasing particle size increases the surface-to-volume ratio, which is expressed as the specific surface area A_{sp}, namely, the area A (cm²) per unit volume V (1 cm³) or per unit mass M (1 gram). For a sphere, $A = 4\pi r^2$ and $V = 4/3\,\pi r^3$. If the density, d, of the material is expressed in g/cm³, the specific surface area is

$$A_{sp} = \frac{A}{V} = \frac{4\pi r^2}{4/3\pi r^3} = \frac{3}{r}\ \text{cm}^2/\text{cm}^3 = \frac{3}{r}\ \text{cm}^{-1}$$

or

$$A_{sp} = \frac{A}{M} = \frac{A}{Vd} = \frac{4\pi r^2}{4/3\pi r^3 d} = \frac{3}{rd}\ \text{cm}^2/\text{g}$$

Table XI—Effect of Comminution on Specific Surface Area of a Volume of $4\pi/3$ cm³, Divided into Uniform Spheres of Radius R

Number of spheres	R	A_{sp} cm²/cm³
1	1 cm	3
10^3	0.1 cm = 1 mm	3×10
10^6	0.1 mm	3×10^2
10^9	0.01 mm = 10 μm	3×10^3
10[illegible]	1 μm	3 × 10[illegible]
10[illegible]	0.1 μm	[illegible] × 10[illegible]
10[illegible]	0.01 μm	3 × 10[illegible]
10[illegible]	10 Å = 1 nm	[illegible] × 10[illegible]
10^{23}	1 Å	3×10^8

Shaded region corresponds to colloidal particle-size range

Table XI illustrates the effect of comminution on the specific surface area of 4 π/3 cm³ of a material consisting initially of one sphere of 1 cm radius. As the material is broken up into an increasingly larger number of smaller and smaller spheres, its specific surface area increases commensurately.

The solid adsorbents activated charcoal and kaolin have specific surface areas of about 6×10^6 cm²/g and 10^4 cm²/g, respectively. One gram of activated charcoal, because of its extensive porosity and internal voids, has an area equal to ⅙ acre.

In conclusion, colloidal systems by definition are those polyphasic systems where at least one dimension of the disperse phase measures between 10 or 100 Å and a few micrometers. The term "colloidal" designates a state of matter characterized by submicroscopic dimensions rather than certain substances. Any dispersed substance with the proper dimension or dimensions is in the colloidal state.

Physical States of Disperse and Continuous Phases

A useful classification of colloidal systems (systems in the colloidal particle size range) is based on the state of matter of the disperse phase and the dispersion medium, ie, whether they are solid, liquid or gaseous.[25,27] Table XII summarizes the various combinations and lists examples. A *sol* is the colloidal dispersion of a solid in a liquid or gaseous medium. Prefixes designate the dispersion medium, such as hydrosol, alcosol, aerosol for water, alcohol and air, respectively. Sols are fluid. If the solid particles form bridged structures possessing some mechanical strength, the system is called a gel (hydrogel, alcogel, aerogel).

Interaction Between Disperse Phase and Dispersion Medium

A second useful classification of colloidal dispersions, originated by Ostwald, is based on the affinity or interaction between the disperse phase and the dispersion medium.[2,3,8] It refers mostly to solid-in-liquid dispersions. According to this classification, colloidal dispersions are divided into the two broad categories of lyophilic and lyophobic. Some soluble, low-molecular-weight substances have molecules with both tendencies, forming a third category called association colloids.

Lyophilic Dispersions—Where there is considerable attraction between the disperse phase and the liquid vehicle, ie, extensive solvation, the system is said to be *lyophilic* (solvent-loving). If the dispersion medium is water, the system is said to be *hydrophilic*. Such solids as bentonite, starch, gelatin, acacia and povidone swell, disperse or dissolve spontaneously in water.

Hydrophilic colloidal dispersions can be subdivided further as follows:

True solutions, formed by water-soluble polymers (acacia and povidone).

Gelled solutions, gels or jellies if the polymers are present at high concentrations and/or at temperatures where their water solubility is low. Examples of such hydrogels are relatively concentrated solutions of gelatin and starch, which set to gels on cooling, or of methylcellulose, which gel on heating.

Particulate dispersions, where the solids do not form molecular solutions but remain as discrete though minute particles. Bentonite and microcrystalline cellulose form such hydrosols.

Lipophilic or oleophilic substances have pronounced affinity for oils. Oils are nonpolar liquids consisting mainly of hydrocarbons, with few polar groups and low dielectric constants. Examples are mineral oil, benzene, carbon tetrachloride, vegetable oils (cottonseed or peanut oil) and essential oils (lemon or peppermint oil). Substances which form *oleophilic* colloidal dispersions include polymers like polystyrene and unvulcanized or gum rubber, which dissolve molecularly in benzene, magnesium or aluminum stearate or which dissolve or disperse in cottonseed oil, and activated charcoal, which forms sols or particulate dispersions in all oils.

Because of the high affinity or attraction between the dispersion medium and the disperse phase, lyophilic dispersions form spontaneously when the liquid vehicle is brought into contact with the solid phase. They are thermodynamically stable and reversible, ie, they are easily reconstituted even after the dispersion medium has been removed from the solid phase.[22,24-27]

Table XII—Classification of Colloidal Dispersions According to State of Matter

Disperse Phase	Dispersion Medium (Vehicle)		
	Solid	Liquid	Gas
Solid	Zinc oxide paste (zinc oxide + starch in petrolatum). Toothpaste (dicalcium phosphate or calcium carbonate with sodium carboxymethylcellulose binder). Pigmented plastics (titanium dioxide in polyethylene).	Sols: Bentonite Magma NF. Trisulfapyrimidines Oral Suspension USP. Magnesia and Alumina Oral Suspension USP. Tetracycline Oral Suspension USP.	Solid aerosols: Smoke, dust. Epinephrine Bitartrate Inhalation Aerosol USP. Isoproterenol Sulfate Inhalation Aerosol.
Liquid	Absorption bases (aqueous medium in Hydrophilic Petrolatum USP). Emulsion bases (oil in Hydrophilic Ointment USP). Butter.	Emulsions: Mineral Oil Emulsion USP. Soybean oil in water emulsion for IV feeding. Milk. Mayonnaise.	Liquid aerosols: Mist, fog. Nasal relief sprays (naphazoline hydrochloride solution). Betamethasone Valerate Topical Aerosol USP. Povidone-Iodine Topical Aerosol.
Gas	Solid foams (foamed plastics and rubbers). Pumice.	Foams. Carbonated beverages. Effervescent salts in water.	No colloidal dispersions.

Lyophobic Dispersions—When there is little attraction between the disperse phase and the dispersion medium, the dispersion is said to be *lyophobic* (solvent-hating). *Hydrophobic* dispersions consist of particles that are not hydrated, so that water molecules interact with or attract one another in preference to solvating the particles. They include aqueous dispersions of oleophilic materials such as polystyrene or gum rubber (latex), steroids and other organic lipophilic drugs, paraffin wax, magnesium stearate, and of cottonseed or soybean oil (emulsion). While lipophilic materials are generally hydrophobic, materials like sulfur, silver chloride and gold form hydrophobic dispersions without being lipophilic. Water-in-oil emulsions are lyophobic dispersions in lipophilic vehicles.

Because of the lack of attraction between the disperse and the continuous phase, lyophobic dispersions are intrinsically unstable and irreversible. Their large surface free energy is not lowered by solvation. The dispersion process does not take place spontaneously, and once the dispersion medium has been separated from the disperse phase, the dispersion is not easily reconstituted. The dividing line between hydrophilic and hydrophobic dispersions is not very sharp. For instance, gelatinous hydroxides of polyvalent metals such as $Al(OH)_3$ and $Mg(OH)_2$, and clays such as bentonite and kaolin, possess some characteristics of both.[22,24,27]

Association Colloids—Organic compounds which contain large hydrophobic moieties together with strongly hydrophilic groups in the same molecule are said to be amphiphilic. While the individual molecules are generally too small to bring their solutions into the colloidal size range, they tend to associate in aqueous or oil solutions into micelles (see above). Because micelles are large enough to qualify as colloidal particles, such compounds are called association colloids.

Lyophobic Dispersions

Most of the discussion of lyophobic dispersions deals with hydrophobic dispersions or hydrosols (hydrophobic solids or liquids dispersed in aqueous media) because water is the most widely used vehicle. They comprise aqueous dispersions of insoluble organic and inorganic compounds which usually have low degrees of hydration. Organic compounds which are preponderantly hydrocarbon in nature and possess few hydrophilic or polar groups are insoluble in water and hydrophobic.

Hydrophobic dispersions are intrinsically unstable. The most stable state of such systems contains the disperse phase coalesced into large crystals or drops, so that the specific surface area and surface free energy are reduced to a minimum. Therefore, mechanical, chemical or electrical energy must be supplied to the system to break up the disperse phase into small particles, providing for the increase in surface free energy resulting from the parallel increase in specific surface area. Furthermore, special means must be found to stabilize hydrophobic dispersions, preventing the otherwise spontaneous coalescence or coagulation of the disperse phase after it has been finely dispersed.

Preparation and Purification of Lyophobic Dispersions

Colloidal dispersions are intermediate in size between true solutions and coarse suspensions. They can be prepared by aggregation of small molecules or ions until particles of colloidal dimensions result (condensation methods), or by reducing coarse particles to colloidal dimensions through comminution or peptization (dispersion methods).

Dispersion Methods—The first method, *mechanical disintegration* of solids and liquids into small particles and their dispersion in a fluid vehicle, is frequently carried out by input of mechanical energy via shear or attrition. Equipment such as colloid and ball mills, micronizers and, for emulsions, homogenizers is described in Chapters 83 and 88 and in Ref 29. Dry grinding with inert, water-soluble diluting agents also produces colloidal dispersions. Sulfur hydrosols may be prepared by triturating the powder with urea or lactose followed by shaking with water.

Ultrasonic generators provide exceptionally high concentrations of energy. Successful dispersion of solids by means of ultrasonic waves can only be achieved with comparatively soft materials such as many organic compounds, sulfur, talcum, and graphite. Where fine emulsions are mandatory, such as soybean oil-in-water emulsions used for intravenous feeding, emulsification by ultrasound waves is the method of choice.[29] The formation of aerosols is described in Chapter 92.

It should be reiterated that hydrosols of hydrophobic substances are intrinsically unstable. While mechanical disintegration may break up the disperse phase into colloidal particles, the resultant dispersions tend towards separation of that phase. Recrystallization, coagulation or coalescence causes the disperse particles to become progressively coarser and fewer, ultimately resulting in the separation of a macroscopic phase. To avoid this, stabilizing agents must be added during or shortly after the dispersion process (see below). For instance, lecithin may be used to stabilize soybean oil emulsions.

Peptization is a second method for preparing colloidal dispersions. The term, coined by Graham, is defined as the breaking up of aggregates or secondary particles into smaller aggregates or into primary particles in the colloidal size range. Particles which are not formed of smaller ones are called "primary." Peptization is synonymous with *deflocculation*. It can be brought about by the removal of flocculating agents, usually electrolytes, or by the addition of deflocculating or peptizing agents, usually surfactants, water-soluble polymers or ions which are adsorbed at the particle surface.[24,27]

The mechanisms of the following examples are explained in subsequent sections. When powdered activated charcoal is added to water with stirring, the aggregated grains are broken up only incompletely and the resultant suspension is gray and translucent. The addition of 0.1% or less of sodium lauryl sulfate or octoxynol disintegrates the grains into finely dispersed particles forming a deep black and opaque dispersion. Ferric or aluminum hydroxide freshly precipitated with ammonia can be peptized with small amounts of acids which reduce the pH below the isoelectric points of the hydroxides (see below). Even washing the gelatinous precipitate of $Al(OH)_3$ with water tends to peptize it. In quantitative analysis, the precipitate is therefore washed with dilute solutions of ammonium salts that act as flocculating agents, rather than with water.

Condensation Methods—The preparation of sulfur hydrosols is employed to illustrate condensation or aggregation methods. Sulfur is insoluble in water but somewhat soluble in alcohol. When an alcoholic solution of sulfur is mixed with water, a bluish white colloidal dispersion results. In the absence of added stabilizing agents, the particles tend to agglomerate and precipitate on standing. This technique of dissolving the material in a water-miscible solvent such as alcohol or acetone and producing a hydrosol by precipitation with water is applicable to many organic compounds, and has been used to prepare hydrosols of natural resins like mastic, of stearic acid and of polymers (the so-called pseudolatexes).

For sulfur, another less common physical method is to introduce a current of sulfur vapor into water. Condensation produces colloidal particles. Alternatively, the very fine powder produced by condensing sulfur vapor on cold

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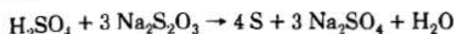
solid surfaces (sublimed sulfur or flowers of sulfur) can be dispersed in water by addition of a suitable surfactant to produce a hydrosol.

Chemical methods include the reaction between hydrogen sulfide and sulfur dioxide, eg, by bubbling H_2S into an aqueous SO_2 solution:

$$2\,H_2S + SO_2 \rightarrow 3\,S + 2\,H_2O$$

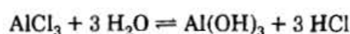
The same reaction occurs when aqueous solutions containing sodium sulfide and sulfite are acidified with an excess of sulfuric or hydrochloric acid. Another reaction is the decomposition of sodium thiosulfate by sulfuric acid, using either very dilute or very concentrated solutions to obtain colloidally dispersed sulfur:



$$H_2SO_4 + 3\,Na_2S_2O_3 \rightarrow 4\,S + 3\,Na_2SO_4 + H_2O$$

Both reactions also produce pentathionic acid, $H_2S_5O_6$, as a by-product. The preferential adsorption of the pentathionate anion at the surface of the sulfur particles confers a negative electric charge on the particles, stabilizing the sol (see below).[22,26,27] When powdered sulfur is boiled with a slurry of lime, it dissolves with the formation of calcium pentasulfide and thiosulfate. Subsequent acidification produces the colloidal "milk of sulfur," which on washing and drying yields Precipitated Sulfur USP (see Chapter 82).

Sols of ferric, aluminum, chromic, stannic and titanium hydroxides or hydrous oxides are produced by hydrolysis of the corresponding chlorides or nitrates:

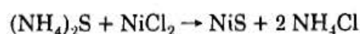


$$AlCl_3 + 3\,H_2O \rightleftharpoons Al(OH)_3 + 3\,HCl$$

Hydrolysis is promoted by boiling the solution and/or by adding a base to neutralize the acid formed.

Double decompositions producing insoluble salts can lead to colloidal dispersions. Examples are silver chloride and nickel sulfide:

$$NaCl + AgNO_3 \rightarrow AgCl + NaNO_3$$



$$(NH_4)_2S + NiCl_2 \rightarrow NiS + 2\,NH_4Cl$$

Compare also the preparation of White Lotion, which contains precipitated zinc sulfide and sulfur (Chapter 63). Reducing salts of gold, silver, copper, mercury, platinum, rhodium and palladium with formaldehyde, hydrazine, hydroxylamine, hydroquinone or stannous chloride produces hydrosols of the metals. These are strongly colored, eg, red or blue.[21,22,27]

Radioactive Colloids—Colloidal dispersions containing radioactive isotopes find increasing diagnostic and therapeutic application in nuclear medicine. Radioactive colloids that accumulate in tumors and/or lesions or emboli, indicating their location and size, may be used as diagnostic aids. Radioactive colloids with a particle size of about 300 Å, injected intravenously, locate mainly in the reticuloendothelial systems of liver, spleen and other organs and are used in scintillation imaging. The radiation emitted by the colloids is made visible by stationary or scanning devices which show the location, size and shape of the organ being investigated, as well as any tumors within. Radiocolloids are useful in anticancer radiation therapy because of their low solubility, radiation characteristics, and their ability to accumulate and remain located in certain target organs or tumors.[30]

Colloidal gold Au 198 is made by reducing a solution of gold (^{198}Au) chloride either by treatment with ascorbic acid or by heating with an alkaline glucose solution. Gelatin is added as a protective colloid (see below). The particle size ranges from 50 to 500 Å with a mean of 300 Å. The color of the sol is cherry-red in transmitted light. Violet or blue sols have excessively large particle sizes and should be discarded. Colloidal gold is used as a diagnostic and therapeutic aid (see Chapter 33). The half-life of ^{198}Au is 2.7 days.

Technetium 99m sulfur colloid is prepared by reducing sodium pertechnetate ^{99m}Tc with sodium thiosulfate. The product, a mixture of technetium sulfide and sulfur in the colloidal particle size range, is stabilized with gelatin. It is used chiefly in liver, spleen and bone scanning. Its half-life is 6.0 hour.

Microspheres of gelatin or human serum albumin can be prepared in fairly narrow particle-size ranges from 100–200 Å through 45–55 μm. A variety of β- and γ-emitting radionuclides such as ^{131}I, ^{99m}Tc, ^{113m}In or ^{51}Cr can be incorporated to label the microspheres. Such products have been used to scan heart, brain, urogenital and gastrointestinal tracts, liver, and in pulmonary perfusion and inhalation studies.[30]

Refer to Chapters 32 and 33 for an in-depth discussion of radioisotopes.

Organic compounds that are weak bases, such as alkaloids, are usually much more soluble at lower pH values where they are ionized than at higher pH values where they exist as the free base. Increasing the pH of their aqueous solutions well above their pKa may cause precipitation of the free base. Organic compounds which are weak acids, such as barbiturates, are usually much more soluble at higher pH values where they are ionized than at lower pH values where they are in the un-ionized acid form. Lowering the pH of their solutions well below their pKa may cause precipitation of the un-ionized acid. Depending on the supersaturation of the un-ionized acids or bases and on the presence of stabilizing agents, the resultant dispersions may be in the colloidal range.

Kinetics of Particle Formation—When the solubility of a compound in water is exceeded, its solution becomes supersaturated and the compound may precipitate or crystallize. The rate of precipitation, the particle size (whether colloidal or coarse), and the particle size uniformity or distribution (whether a narrow distribution and nearly monodisperse or homodisperse particles, or a broad distribution and polydisperse or heterodisperse particles) depend on two successive and largely independent processes, nucleation and growth of nuclei.

When a solution of a salt or of sucrose is supercooled, or when a chemical reaction produces a salt in a concentration exceeding its solubility product, separation of the excess solid from the supersaturated solution is far from instantaneous. Clusters of ions or molecules called nuclei must exceed a critical size before they become stable and capable of growing into colloidal size crystals. These embryonic particles have much more surface for a given weight of material than large and stable crystals, resulting in higher surface free energy and greater solubility.

Whether *nucleation* takes place depends on the *relative supersaturation.* If C is the actual concentration of the solute before crystallization has set in, and C_s is its solubility limit, $C - C_s$ is the supersaturation and $(C - C_s)/C_s$ is the relative supersaturation. Von Weimarn recognized that the rate or velocity of nucleation (number of nuclei formed per liter per second) is proportional to the relative supersaturation. Nucleation seldom occurs at relative supersaturations below 3. The foregoing statement refers to homogeneous nucleation, where the nuclei are clusters of the same chemical composition as the crystallizing phase. If the solution contains solid impurities, such as dust particles in suspension, these may act as nuclei or centers of crystallization (heterogeneous nucleation).

Once nuclei have formed, the second process, *crystallization,* begins. Nuclei grow by accretion of ions or molecules from solution forming colloidal or coarser particles until the supersaturation is relieved, ie, until $C = C_s$. The rate of

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crystallization or growth of nuclei is proportional to the supersaturation. The appropriate equation,

$$\frac{dm}{dt} = \frac{A_{sp}D}{\delta}(C - C_s)$$

is similar to the Noyes-Whitney equation governing the dissolution of particles (see Chapter 31) except that $C < C_s$ for the latter process, making dm/dt negative. In both equations, m is the mass of material crystallizing out in time t, D is the diffusion coefficient of the molecules or ions of the solute, δ is the length of the diffusion path or the thickness of the liquid layer adhering to the growing particles, and A_{sp} is their specific surface area. The presence of dissolved impurities may affect the rate of crystallization and even change the crystal habit, provided that these impurities are surface-active and become adsorbed on the nuclei or growing crystals.[22,23,25-28] For instance, 0.005% polysorbate 80 or octoxynol 9 significantly retard the growth of methylprednisolone crystals in aqueous media. Gelatin or povidone, at concentrations <0.10%, retard the crystal growth of sulfathiazole in water.

Von Weimarn found that the particle size of the crystals depends strongly on the concentration of the precipitating substance. At a very low concentration and slight relative supersaturation, diffusion is quite slow because the concentration gradient is very small. Sufficient nuclei will usually form to relieve the slight supersaturation locally. Crystal growth is limited by the small amount of excess dissolved material available to each particle. Hence, the particles cannot grow beyond colloidal dimensions. This condition is represented by points A, D and G of the schematic plot of von Weimarn (Fig 19-20). At intermediate concentrations, the extent of nucleation is somewhat greater but much more material is available for crystal growth. Coarse crystals rather than colloidal particles result (points B, E or H).

At high concentrations, nuclei appear so quickly and in such large numbers that supersaturation is relieved almost immediately, before appreciable diffusion occurs. The high viscosity of the medium also slows down diffusion of excess dissolved ions or molecules, retarding crystal growth without substantially affecting the rate of nucleation. A large number of very small particles results which, because of their proximity, tend to link, producing a translucent gel (points C and F). On subsequent dilution with water, such gels usually yield colloidal dispersions.

Thus, colloidal systems are usually produced at very low and high supersaturations. Intermediate values of supersaturation tend to produce coarse crystals. Low solubility is a necessary condition for producing colloidal dispersions. If the solubility of the precipitate is increased, for instance by heating the dispersion, a new family of curves will result, similar in shape to ABC, DEF, and GHI of Fig 19-20, but displaced upwards (towards larger particle sizes) and to the right (towards higher concentrations).[25-28]

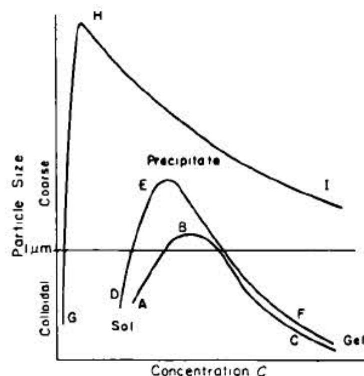


Fig 19-20. Effect of the concentration of the precipitating material and of aging on particle size.[28] Curves ABC, DEF and GHI correspond to increasing aging. Both axes are on a logarithmic scale.

Condensation methods generally produce polydisperse sols because nucleation continues while established nuclei grow. The particles in the resultant dispersion grew from nuclei formed at different times and had different growth periods.

A useful technique for preparing monodispersed sols in the colloidal range by precipitation consists in forming all the nuclei in a single, brief burst: When, in the course of the precipitation process, the rate of homogeneous nucleation becomes appreciable, a brief period of nucleation relieves the supersaturation partially to such an extent that no new nuclei form subsequently. By controlling the precipitation process, it is rendered so slow that the supersaturation remains too small for further nucleation. Therefore, the nuclei formed in the initial burst grow uniformly by diffusion of the precipitating material as the precipitation process proceeds slowly. Throughout the rest of the precipitation, the supersaturation never again reaches sufficiently high values for forming new nuclei. It is relieved by continuous growth of the existing nuclei.[23,25,31]

Controlled hydrolysis of salts of di- and trivalent cations in aqueous solution at elevated temperatures has been used to produce colloidal dispersions of metal (hydrous) oxides of uniform size and shape, in a variety of well-defined shapes (eg, sphere, lath, cube, disc, hexagonal). Complexation of the cations, concentration and temperature control the rate of hydrolysis and, hence, the chemical composition, crystallinity, shape and size of the dispersed phase.[32]

A feature of Fig 19-20 is that aging increases the particle size. Curves ABC, DEF and GHI correspond to increasing times after mixing the reagents. Typical ages are 10–30 min, several hours, and weeks or years, respectively. This gradual increase in particle size of crystals in their mother liquor is a recrystallization process called *Ostwald ripening*. Very small particles have a higher solubility than large particles of the same substance owing to their greater specific surface area and higher surface free energy. In a saturated solution containing precipitated particles of the solute in a wide range of particle sizes, the very smallest particles dissolve spontaneously and the material deposits onto the large particles. The growth of the large crystals at the expense of the very small ones occurs because this process lowers the free energy of the dispersion. As mentioned above, the most stable system is the suspension of a few coarse crystals, whereas the colloidal dispersion of a great many fine particles of the same substance is intrinsically less stable.

The spontaneous coarsening of colloidal dispersions on aging is accelerated by a relatively high solubility of the precipitate and can be retarded by lowering the solubility or by adding traces of surface-active compounds which are adsorbed at the particle surface. For instance, barium sulfate precipitated by mixing concentrated solutions of sodium sulfate and barium chloride is largely in the colloidal range and passes through filter paper. The colloidal particles gradually grow in size by Ostwald ripening, forming large crystals which can be removed quantitatively by filtration. Heating the aqueous dispersion speeds up this recrystallization by increasing the solubility of barium sulfate in water. The addition of ethyl alcohol lowers the solubility, retarding Ostwald ripening so that the dispersion remains in the colloidal state for years.

Mathematically the effect of particle size on solubility is expressed as

$$S = S_\infty \exp\left(\frac{2\gamma M}{r\rho RT}\right) \quad (34)$$

Table XIII—Effects of Particle Size on Solubility

r (μm)	S
0.01	$7 S_\infty$
0.10	$1.12 S_\infty$
1.0	$1.01 S_\infty$
10	$1.001 S_\infty$

$M = 500$; $\gamma = 30$ ergs/cm^2; $\rho = 1$

where S is the solubility of a spherical crystal of radius r, S_∞ is the solubility of an infinitely large crystal ($r = \infty$), M is the molecular weight, ρ is the density, γ is the crystal/solvent interfacial tension, R is the gas constant and T is the absolute temperature. Only approximations can be obtained with this equation because the particles are not spheres, and γ values are different for different crystal faces. Table XIII shows the magnitude of particle size effects on the solubility for reasonable values of M, γ and ρ. It is evident that with particles in the colloidal range, ie, $r \geq 1$ μm, S values become appreciably greater than that for a coarse crystal, hence the tendency for very fine particles to dissolve and for coarse crystals to grow at the expense of the former. This difference in solubility explains why difficulty is encountered in preparing and stabilizing suspensions of very fine particles of certain substances.

Two techniques are used to increase the solubility of very slightly soluble drugs and, hence, their rate of dissolution *in vivo*. Many organic compounds exist in various polymorphic modifications. For instance, corticosterone, testosterone, sulfaguanidine and pentobarbital each have four polymorphic forms, with different melting points and crystal structures. The three metastable polymorphs have higher solubilities than the stable form. Solvates of solid drugs, eg, hydrates, have different crystalline structures and either higher or lower solubilities than the anhydrous forms. Theophylline monohydrate is less soluble than the anhydrous form while succinylsulfathiazole is less soluble than its solvate with 1-pentanol. Milling and grinding organic crystals may produce significant proportions of amorphous or strained crystalline material, which has higher solubility than the original crystalline material.[33]

Another process by which particles in colloidal dispersions grow in size is by agglomeration of individual particles into aggregates. This process, called coagulation, is discussed below.

Purification of Hydrosols by Dialysis and Ultrafiltration

Many hydrosols contain low molecular-weight, water-soluble impurities. Inorganic dispersions often contain salts formed by the reaction producing the disperse phase. Salts are especially objectionable in the case of hydrophobic dispersions because they tend to coagulate such dispersions. Protein solutions often contain salts added as part of the separation procedure. The blood of patients with renal insufficiency contains excessive concentrations of urea and other low-molecular-weight metabolites and salts. These dissolved impurities of small molecular size are removed from the colloidal dispersions by means of membranes with pore openings smaller than the colloidal particles.

Membranes—Conventional filter papers are permeable to colloidal particles as well as to small solute molecules. Among the early membranes capable of retaining colloidal particles but permeable to small solute molecules were pig's bladder and parchment. Most membranes in current use consist of cellulose, cellulose nitrate prepared from collodion, cellulose acetate or synthetic polymers, and are available in a variety of shapes, gauges, and pore sizes. *Gel cellophane* is most widely used. It consists of sheets or tubes of cellulose made by extruding cellulose xanthate solutions (viscose) through slit or annular dies into a sodium bisulfate/sulfuric acid bath which decomposes the xanthate, precipitating the regenerated cellulose in a highly swollen or gel state. If the cellulose film were permitted to dry after purification and washing with water, it would crystallize and shrink excessively, losing most of its extensive micropore structure and turning somewhat brittle. The film is therefore impregnated with glycerin before drying. Glycerin remains in the film rather than evaporating like water. It reduces the shrinkage and blocks crystallization. This action prevents the collapse of the porous gel structure and plasticizes the film, keeping it flexible. A typical dialysis tube made from sausage casing swells to about twice its thickness in water and has an average pore diameter of 34 Å. While the pore structure of cellophane films used in dialysis and ultrafiltration causes retention of colloidal particles but permits the passage of small solute molecules, osmotic membranes are only permeable to water and retain small solute molecules as well as colloidal particles.

Dialysis—The colloidal dispersion is placed inside a sac made of sausage casing dipping in water. The small solute molecules diffuse out into the water while the colloidal material remains trapped inside because of its size. The rate of dialysis is increased by increasing the area of the membrane, by stirring, and by maintaining a high concentration gradient across the membrane. For the latter purpose, the water is replenished continuously or at least frequently. A membrane configuration which provides a particularly extensive transfer area for a given volume of dispersion is the hollow fiber. A typical fiber measures 175 μm inside diameter and 225 μm outside diameter. The dispersion to be dialyzed is circulated inside a bundle of parallel fibers while water is circulated outside the fibers throughout the bundle. Dialysis of the diffusing species takes place across the thin fiber wall. Dialysis is used in the laboratory to purify sols and to study binding of drugs by proteins, as well as in some manufacturing processes.

Electrodialysis—If the low-molecular-weight impurities to be removed are electrolytes, the dialysis can be speeded up by applying an electric potential to the sol which produces electrolysis. An electrodialyzer (Fig 19-21) is divided into three compartments by two dialysis membranes supported by screens. The two outer compartments, in which the two electrodes are placed, are filled with water while the sol is placed into the center compartment. Under the influence of the applied potential, the anions migrate from the sol into the anode (right) compartment while the cations migrate into the cathode compartment. Low-molecular-weight nonelectrolyte solutes diffuse into either compartment.

Colloidal particles are usually charged and therefore tend to migrate towards the membrane sealing off the compartment with the electrode of opposite charge. The combination of electrophoresis (see below) and gravitational sedimentation produces the accumulation of negatively charged sol particles shown in Fig 19-21. Hence the supernatant

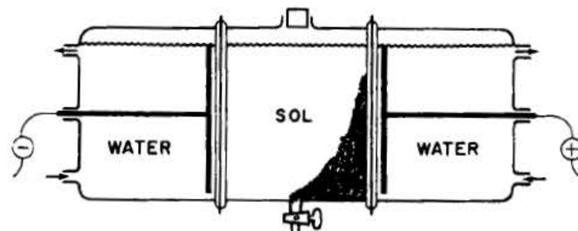


Fig 19-21. Electrodialyzer showing electrodecantation

liquid can be changed by decantation. This process, which may be used to speed up electrodialysis, is called *electrodecantation.*[21,25]

Ultrafiltration—When a sol is placed in a compartment closed by a dialysis membrane and pressure is applied, the liquid and the small solute molecules are forced through the membrane while the colloidal particles are retained. This process, called ultrafiltration, is based on a sieving mechanism in which all components smaller than the pore size of the filter membrane pass through it. The pressure difference required to push the dispersion medium through the ultrafilter is provided by gas pressure applied on the sol side or by suction on the filtrate side. The membrane is usually supported on a fine wire screen.[24-27]

As ultrafiltrate is being removed, the sol becomes more concentrated because a constant amount of disperse particles is confined to a decreasing volume of liquid. Some dissolved small molecules or ions are left in the sol together with the residual water. To avoid the increase in concentration of the colloidal particles and remove the dissolved impurities completely, the ultrafiltrate squeezed from the sol is replenished continuously or intermittently with an equal volume of water. During ultrafiltration, solids tend to accumulate on and near the membrane. To prevent this buildup and maintain uniform composition throughout the sol, it is stirred.

Bundles of hollow fibers are used for ultrafiltration in the laboratory and on large scale. To withstand higher pressures, the wall thickness of the fibers used in ultrafiltration is usually greater than that of fibers used exclusively for dialysis. When hollow fibers are fouled by excessive accumulation of solids on the inner wall, they are cleaned by backflushing with water or ultrafiltrate.

Hemodialysis—The blood of uremic patients is dialyzed periodically in "artificial kidney" dialyzers to remove urea, creatinine, uric acid, phosphate and other metabolites, and excess sodium and potassium chloride. The dialyzing fluid contains sodium, potassium, calcium, chloride and acetate ions (the latter are converted in the body to bicarbonate), dextrose and other constituents in the same concentration as normal plasma. Since it contains no urea, creatinine, uric acid, phosphate nor any of the other metabolites normally eliminated by the kidneys, these compounds diffuse from the patient's blood into the dialyzing fluid until their concentration is the same in blood and fluid. Sodium and potassium chloride diffuse from blood to fluid because of their higher initial concentration in the blood, and continue to diffuse until the concentration is equalized. The volume of dialyzing fluid is much greater than that of blood. The great disparity in volume and the replenishment of dialyzate with fresh fluid ensure that the metabolites and the excess of electrolytes are removed almost completely from the blood. Hemodialysis is also employed in acute poisoning cases.

Plasma proteins and blood cells cannot pass through the dialysis membrane because of their size. Edema resulting from water retention can be relieved by ultrafiltration through the application of a slight pressure on the blood side or a partial vacuum on the fluid side.

The three geometries used to circulate the blood and the dialyzing fluid in a countercurrent fashion are a coil of flattened cellulose tubing wound concentrically with a supporting mesh screen around a core, a stack of flat cellulose sheets separated by ridged or grooved plates, and hollow fibers. The regenerated cellulose used in the former two is precipitated from a cuprammonium solution. The hollow cellulose acetate fibers have an outside diameter of about 270 μm and a wall thickness of 30 μm.[34] The advantage of hollow fibers is their compactness. A bundle of 10,000 fibers 18 cm long has a surface area of 1.4 m^2.

Particle Shape, Optical, and Transport Properties of Lyophobic Dispersions

Hydrophobic materials handled by pharmacists in aqueous dispersion range from metallic conductors to inorganic precipitates to organic solids and liquids which are electric insulators. Despite the great diversity of the hydrophobic disperse phase, their hydrosols have certain common characteristics.

Particle Shape and Particle Size Distribution—Both of these properties depend on the chemical and physical nature of the disperse phase and on the method employed to prepare the dispersion. Primary particles exist in a great variety of shapes. Their aggregation produces an even greater variety of shapes and structures. Precipitation and mechanical comminution generally produce randomly shaped particles unless the precipitating solids possess pronounced crystallization habits or the solids being ground possess strongly developed cleavage planes. Precipitated aluminum hydroxide gels and micronized particles of sulfonamides and other organic powders have typical irregular random shapes. An exception is bismuth subnitrate. Even though its particles are precipitated by hydrolyzing bismuth nitrate solutions with sodium carbonate, its particles are lath-shaped. Precipitated silver chloride particles have a cubic habit which is apparent under the electron microscope. Lamellar or plate-like solids in which the molecular cohesion between layers is much weaker than within layers frequently preserve their lamellar shape during mechanical comminution, because milling and micronization break up stacks of thin plates in addition to fragmenting plates in the lateral dimensions. Examples are graphite, mica and kaolin. Figure 19-22 shows a Georgia crude clay as mined. Processing yields the refined, fine-particle kaolinite of Fig 19-23. Similarly, macroscopic asbestos and cellulose fibers consist of bundles of microscopic and submicroscopic fibrils. Mechanical comminution or beating splits these bundles into the component fibrils of very small diameters as well as cutting them shorter.

Microcrystalline cellulose is a fibrous thickening agent and tablet additive made by selective hydrolysis of cellulose.

Fig 19-22. Scanning electron micrograph of a crude kaolin clay as mined. Processing yields the fine particle material of Fig 19-23 (courtesy, John L Brown, Engineering Experiment Station, Georgia Institute of Technology).

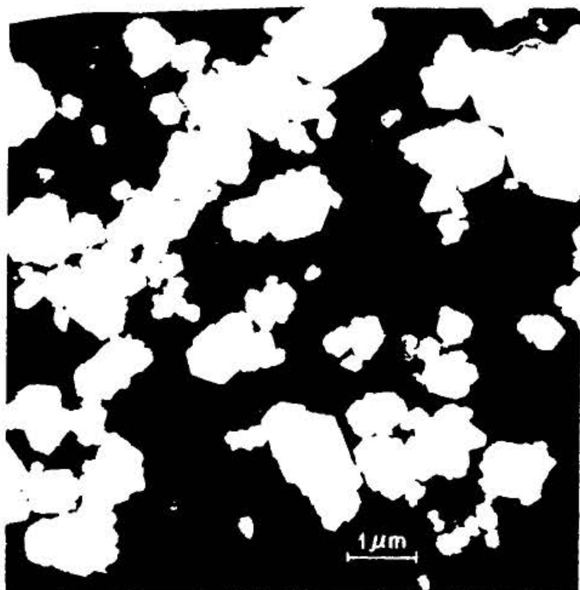


Fig 19-23. Transmission electron micrograph of a well crystallized, fine-particle kaolin. Note hexagonal shape of the clay platelets (courtesy, John L Brown, Engineering Experiment Station, Georgia Institute of Technology).

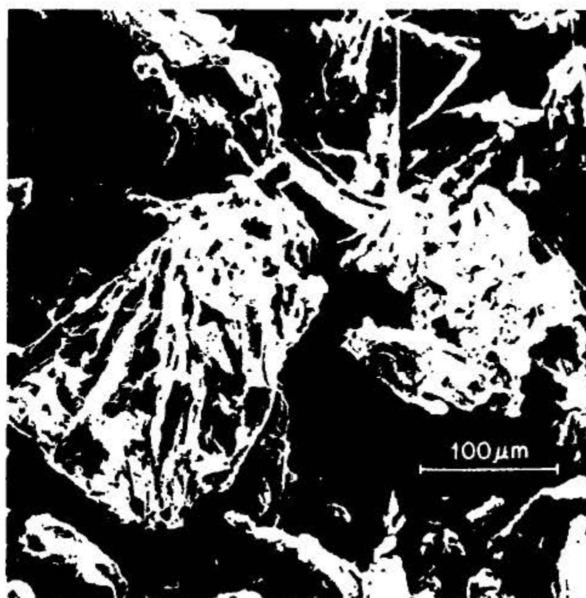


Fig 19-24. Scanning electron micrograph of Avicel PH-102 tableting grade microcrystalline cellulose. The aggregates of fiber bundles are porous and compressible (courtesy, FMC Corporation; Avicel is a registered trademark of FMC Corporation).

Native cellulose consists of crystalline regions where the polymer chains are well aligned and in registry, with maximum interchain attraction by secondary valence forces, called crystallites, and of more disordered regions having lower density and reduced interchain attraction and crystallinity, the so-called "amorphous" regions. During treatment with dilute mineral acid, the acid penetrates the amorphous regions relatively fast and hydrolyzes the polymer chains into water-soluble fragments. If the acid is washed out before it penetrates the crystalline regions appreciably, the crystallites remain intact. Wet milling and spray-drying the aqueous suspension produces spongy and porous aggregates of rod-shaped or fibrillar bundles shown in Fig 19-24. These aggregates, averaging 100 μm in size, were embrittled by the acid treatment and lost the elasticity of the native cellulose. They are well compressible and capable of undergoing plastic deformation, a property important in tableting. Their porosity permits the aggregates to absorb liquid ingredients while still remaining a free-flowing powder, thus preventing these liquids from reducing the flowability of the granulation or direct-compression mass during tableting. The swelling of the cellulosic particles in water speeds up the disintegration of the ingested tablets.

Additional shear breaks up the aggregated bundles into the individual, needle- or rod-shaped cellulose crystallites shown in Fig 19-25. The latter, which average 0.3 μm in length and 0.02 μm in width, are of colloidal dimensions. These primary particles act as suspending agents in water, producing thixotropic structured vehicles. At concentrations above 10%, eg 14 or 15%, the cellulose microcrystals gel water to ointment consistency by swelling and producing a continuous network of rods extending throughout the entire vehicle. Attraction between the elongated particles is presumably due to flocculation in the secondary minimum (see below). Treatment of the microcrystalline mass with sodium carboxymethylcellulose facilitates its disintegration into the primary needle-shaped particles and enhances their thickening action.

While in the special cases of certain clays and cellulose, comminution produces lamellar and fibrillar particles, respectively, as a rule regular particle shapes are produced by

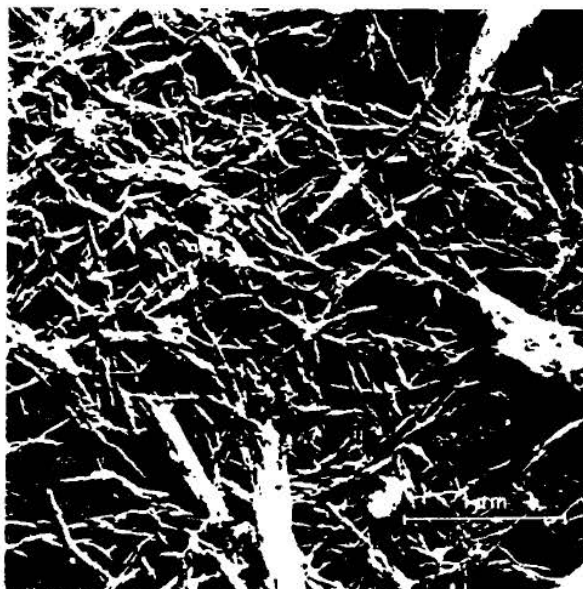


Fig 19-25. Transmission electron micrograph of Avicel RC-591 thickening grade microcrystalline cellulose. The needles are individual cellulose crystallites; some are aggregated into bundles (courtesy, FMC Corporation; Avicel is a registered trademark of FMC Corporation).

condensation rather than by disintegration methods. *Colloidal silicon dioxide* is called fumed or pyrogenic silica because it is manufactured by high-temperature, vapor-phase hydrolysis of silicon tetrachloride in an oxy-hydrogen flame, ie, a flame produced by burning hydrogen in a stream of oxygen. The resultant white powder consists of submicroscopic spherical particles of rather uniform size (narrow particle size distribution). Different grades are produced by different reaction conditions. Relatively large, single

spherical particles are shown in Fig 19-26. Their average diameter is 50 nm (500 Å), corresponding to the comparatively small specific surface area of 50 m^2/g. Smaller spherical particles have correspondingly larger specific surface areas; the grade with the smallest average diameter, 5 nm, has a specific surface area of 380 m^2/g. During the manufacturing process, the finer-grade particles tend to sinter or grow together into chain-like aggregates resembling pearl necklaces or streptococci (see Fig 19-27).

Since fumed silica is amorphous, its inhaled dust causes no silicosis. The spheres of colloidal silicon dioxide are nonporous. While the density of the spherical particles is 2.13 g/cm^3, the bulk density of their powder is a mere 0.05 g/cm^3; the powder is extremely light. This results in two pharmaceutical and cosmetic applications for colloidal silicon dioxide. It is used to increase the fluffiness or bulk volume of powders. Even more than microcrystalline cellulose, the high porosity of silica enables it to absorb a variety of liquids from fluid fragrances to viscous tars, transforming them into free-flowing powders that can be incorporated into tablets or capsules. The porosity in colloidal silicon dioxide is due entirely to the enormous void space between the particles, which themselves are solid.

When these ultrafine particles are incorporated at levels as low as 0.1 to 0.5% into a powder consisting of coarse particles or granules, they coat the surface of the latter and act as tiny ball bearings and spacers, improving the flowability of the powder and eliminating caking. This action is important in tableting. Moreover, colloidal silicon dioxide improves tablet disintegration.

The surface of the particles contains siloxane (Si—O—Si) and silanol (Si—OH) groups. When colloidal silicon dioxide

Fig 19-27. Transmission electron micrograph of Aerosil 130, ground and dusted on. The spheres are fused together into chain-like aggregates (courtesy, Degussa AG of Hanau, West Germany; Aerosil is a registered trademark of Degussa). The suffix 130 gives the specific surface area in m^2/g.

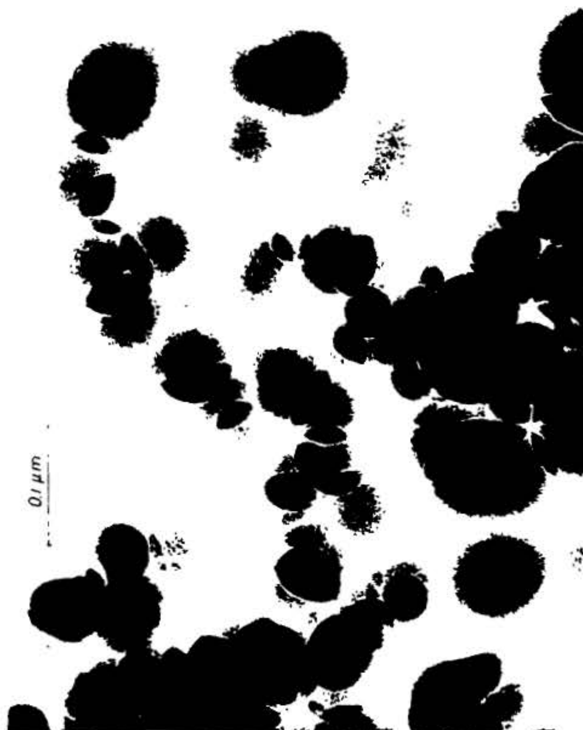


Fig 19-26. Transmission electron micrograph of Aerosil OX 50, ground and dusted on. The spheres are translucent to the electron beam, causing overlapping portions to be darker owing to increased thickness (courtesy, Degussa AG of Hanau, West Germany; Aerosil is a registered trademark of Degussa). The suffix 50 indicates the specific surface area in m^2/g.

powder is dispersed in nonpolar liquids, the particles tend to adhere to one another by hydrogen bonds between their surface groups. With finer grades of colloidal silicon dioxide, the spherical particles are linked together into short chain-like aggregates as shown in Fig 19-27, thus agglomerating into loose three-dimensional networks which increase the viscosity of the liquid vehicles very effectively at levels as low as a few percent. These hydrogen-bonded structures are torn apart by stirring but rebuilt while at rest, conferring thixotropy to the thickened liquids.

The grades which consist of relatively large and unattached spherical particles, such as those of Fig 19-26, are less efficient thickening agents as they lack the high specific surface area and the asymmetry of the finer grades, which consist of short chains of fused spherical particles. In the latter category is Aerosil 200, the grade most widely used as a pharmaceutical adjuvant, whose primary spheres, which are extensively sintered together, have an average diameter of 12 nm. At levels of 8 to 10%, it thickens liquids of low polarity such as vegetable and mineral oils to the consistency of ointments, imparting considerable yield values to them. The consistency of ointments thickened with colloidal silicon dioxide is not appreciably reduced at higher temperatures. Incorporation of colloidal silicon dioxide into ointments and pastes, such as those of zinc oxide, also reduces the syneresis or *bleeding* of the liquid vehicles.

Hydrogen-bonding liquids like alcohols and water solvate the silica spheres, reducing the hydrogen bonding between particles. These solvents are gelled at silica levels of 12–18% or higher.

Latexes of polymers are aqueous dispersions prepared by emulsion polymerization. Their particles are spherical because polymerization of solubilized liquid monomer takes

place inside spherical surfactant micelles which swell because additional monomer keeps diffusing into the micelles. Examples include latex-based paints. Some *clays* grow as plate-like particles possessing straight edges and hexagonal angles, eg bentonite and kaolin (see Fig 19-23). Other clays have lath-shaped (nontronite) or needle-shaped particles (attapulgite).

Emulsification produces spherical droplets to minimize the oil-water interfacial area. Cooling the emulsion below the melting point of the disperse phase freezes it in the spherical shape. For instance, paraffin can be emulsified in 80° water; cooling to room temperature produces a hydrosol with spherical particles.

Sols of viruses and globular proteins, which are hydrophilic, contain compact particles possessing definite geometric shapes. Poliomyelitis virus is spherical, tobacco mosaic virus is rod-shaped, while serum albumin and the serum globulins are prolate ellipsoids of revolution (football-shaped).

Dispersion methods produce sols with wide particle size distributions. Condensation methods *may* produce essentially monodisperse sols provided specialized techniques are employed. Monodisperse polystyrene latexes are available for calibration of electron micrographs (see Fig 19-23). Biologic hydrophilic polymers, such as nucleic acids and proteins, form largely monodisperse particles, as do more highly organized structures such as lipoproteins and viruses.

Light-Scattering by Colloidal Particles—The optical properties of a medium are determined by its refractive index. When the refractive index is uniform throughout, light will pass the medium undeflected. Whenever there are discrete variations in the refractive index caused by the presence of particles or by small-scale density fluctuations, part of the light will be scattered in all directions. An optical property characteristic of colloidal systems, called the *Tyndall beam*, is familiar to everyone in the case of aerosols. When a narrow beam of sunlight is admitted through a small hole into a darkened room, the presence of the minute dust particles suspended in air is revealed by bright flashing points.

A beam of light striking a particle polarizes the atoms and molecules of that particle, inducing dipoles which act as secondary sources and reemit weak light of the same wavelength as the incident light. This phenomenon is called *light-scattering*. The scattered radiation propagates in all directions away from the particle. In a bright room, the light scattered by the dust particles is too weak to be noticeable.

Colloidal particles suspended in a liquid also scatter light. When an intense, narrowly defined beam of light is passed through a suspension, its path becomes visible because of the scattering of light by the particles in the beam. This Tyndall beam becomes most visible when viewed against a dark background in a direction perpendicular to the incident beam. The magnitude of the turbidity or opalescence depends on the nature, size and concentration of the particles. When clear mineral oil is dispersed in an equal volume of a clear aqueous surfactant solution, the resultant emulsion is milky white and opaque due to light scattering. Microemulsions, where the emulsified droplets are about 40 nm (400 Å) in diameter, ie, much smaller than the wavelength of visible light, are transparent and clear to the naked eye.

The *dark-field microscope* or *ultramicroscope*, which permits observation of particles much smaller than the wavelength of light, was the only means of detecting submicroscopic particles before the advent of electron microscopy. A special cardioid condenser produces a hollow cylinder of light and converges it into a hollow cone focused on the sample. The sample is at the apex of the cone, where the light intensity is high. After passing through the sample, the cone of light diverges and passes outside of the microscope objective. A homogeneous sample thus gives a dark field. A similar effect can be produced with a regular Abbe condenser outfitted with a central stop and a strong light source. Colloidal particles scatter light in all directions. Some of the scattered light enters the objective and shows up the particles as bright spots. Thus, even particles smaller than the wavelength of light can be detected, provided their refractive index differs sufficiently from that of the medium. Dissolved polymer molecules and highly solvated gel particles do not scatter enough light to become visible. Asymmetric particles like flat bentonite platelets give flashing effects as they rotate in Brownian motion, because they scatter more light with their basal plane perpendicular to the light beam than edgewise. Brownian motion, sedimentation, electropboretic mobility, and the progress of flocculation can be studied with the dark-field microscope. Polydispersity can be estimated qualitatively because larger particles scatter more light and appear brighter. The resolving power of the ultramicroscope is no greater than that of the ordinary light microscope. Particles closer together than 0.2 μm appear as a single blur.

Turbidity may be used to measure the concentration of dispersed particles in two ways. In *turbidimetry*, a spectrophotometer or photoelectric colorimeter is used to measure the intensity of the light transmitted in the incident direction. Turbidity, τ, is defined by an equation analogous to Beer's law for the absorption of light (see Chapter 30),[24,25,27] namely

$$\tau = \frac{1}{l} \ln \frac{I_0}{I_t}$$

where I_0 and I_t are the intensities of the incident and transmitted light beams, and l is the length of the dispersion through which the light passes.

If the dispersion is less turbid, the intensity of light scattered at 90° to the incident beam is measured with a *nephelometer*. Both methods require careful standardization with suspensions containing known amounts of particles similar to those to be measured. The concentration of colloidal dispersions of inorganic and organic compounds and of bacterial suspensions can thus be measured by their turbidity.

The turbidity or Tyndall effect of hydrophilic colloidal systems like aqueous solutions of gums, proteins and other polymers is far weaker than that of lyophobic dispersions. These solutions appear clear to the naked eye. Their turbidity can be measured with a photoelectric cell/photomultiplier tube and serves to determine the molecular weight of the solute.

The theory of light scattering was developed in detail by Lord Rayleigh. For white nonabsorbing nonconductors or dielectrics like sulfur and insoluble organic compounds, the equation obtained for spherical particles whose radius is small compared to the wavelength of light λ is[24–27]

$$I_s = I_0 \frac{4\pi^2 n_0^2 (n_1 - n_0)^2}{\lambda^4 d^2 c} (1 + \cos^2 \theta)$$

I_0 is the intensity of the unpolarized incident light; I_s is the intensity of light scattered in a direction making an angle θ witb the incident beam and measured at a distance d. The scattered light is largely polarized. The concentration c is expressed as the number of particles per unit volume. The refractive indices n_1 and n_0 refer to the dispersion and the solvent, respectively.

Since the intensity of scattered light is inversely proportional to the fourth power of the wavelength, blue light ($\lambda \cong$ 450 nm or 4500 Å) is scattered much more strongly than red light ($\lambda \cong$ 650 nm or 6500 Å). With incident white light, colloidal dispersions of colorless particles appear blue when

viewed in scattered light, ie, in lateral directions such as 90° to the incident beam. Loss of the blue rays due to preferential scattering leaves the transmitted light yellow or red. Preferential scattering of blue radiation sideways accounts for the blue color of the sky, sea, cigarette smoke, and diluted milk and for the yellow-red color of the rising and setting sun viewed head-on.

The particles in pharmaceutical suspensions, emulsions and lotions are generally larger than the wavelength of light λ. When the particle size exceeds $\lambda/20$, destructive interference between light scattered by different portions of the same particle lowers the intensity of scattered light and changes its angular dependence. Rayleigh's theory was extended to large and to strongly absorbing and conducting particles by Mie and to nonspherical particles by Gans.[21,22,24–27] By using appropriate precautions in experimental techniques and in interpretation, it is possible to determine an average particle size and even the particle size distribution of colloidal dispersions and coarser suspensions by means of turbidity measurements.

Diffusion and Sedimentation—The molecules of a gas or liquid are engaged in a perpetual, random thermal motion which causes them to collide with one another and with the container wall billions of times per second. Each collision changes the direction and the velocity of the molecules involved. Dissolved molecules and suspended colloidal particles are continuously and randomly buffeted by the molecules of the suspending medium. This random bombardment imparts to solutes and particles an equally unceasing and erratic movement called *Brownian motion*, after the botanist Robert Brown who first observed it under the microscope with an aqueous pollen suspension. The Brownian motion of colloidal particles mirrors on a magnified scale the random movement of the molecules of the liquid or gaseous suspending medium, and represents a three-dimensional random walk.

Solute molecules and suspended colloidal particles undergo rotational and translational Brownian movement. For the latter, Einstein derived the equation

$$\bar{x} = \sqrt{2Dt}$$

where $\bar{x}$ is the mean displacement in the x-direction in time t and D is the *diffusion coefficient*. Einstein also showed that for spherical particles of radius r under conditions specified in Chapter 20 for the validity of Stokes' law and Einstein's law of viscosity

$$D = \frac{RT}{6\pi\eta rN}$$

where R is the gas constant, T the absolute temperature, N Avogadro's number, and η the viscosity of the suspending medium.

The diffusion coefficient is a measure of the mobility of a dissolved molecule or suspended particle in a liquid medium. Representative values at room temperature, in cm^2/sec, are 4.7×10^{-6} for sucrose and 6.1×10^{-7} for serum albumin in water. With a diffusion coefficient of 1×10^{-7} cm^2/sec, Brownian motion causes a particle to move by an average distance of 1 cm in one direction in 58 days, by 1 mm in 14 hr, and by 1 μm in 0.05 sec. Smaller molecules diffuse faster in a given medium. Assuming spherical shape, the radius of a serum albumin molecule is 35 Å and that of a sucrose molecule 4.4 Å. The ratio of the radii of the two molecules 35/4.4 = 7.9, is nearly identical with the inverse ratio of their diffusion coefficients in water, $4.7 \times 10^{-6}/6.1 \times 10^{-7} = 7.7$, in agreement with the above equation. Diffusion coefficients of steroids and other molecules of similar size dissolved in absorption bases based on petrolatum are generally in the 10^{-10} to 10^{-8} cm^2/sec range. Steroids have only slightly higher molecular weights than sucrose. Their much smaller diffusion coefficients are due to the much higher viscosity of the vehicle.

Dynamic light-scattering or photon-correlation spectroscopy is based on the fact that the light scattered by particles in Brownian motion undergoes a minute shift in wavelength by the usual Doppler effect. The shift is so small that it can be detected only by laser light beams, which are strictly monochromatic and very intense. The wavelength shift, which shows up as line broadening, is used to determine the diffusion coefficient of the particles,[23,26] which in turn yields their radius according to the equation above.

Brownian motion and convection currents maintain dissolved molecules and small colloidal particles in suspension indefinitely. As the particle size and r increase, the Brownian motion decreases; $\bar{x}$ is proportional to $r^{-1/2}$. Provided that the density of the particle d_P and of the liquid vehicle d_L are sufficiently different, larger particles have a greater tendency to settle out when $d_P > d_L$ or to rise to the top of the suspension when $d_P < d_L$ than smaller particles of the same material.

The rate of *sedimentation* is expressed by the Stokes' equation (Eq 35), which can be rewritten as

$$h = \frac{2(d_P - d_L)r^2gt}{9\eta}$$

where h is the height through which a spherical particle settles in time t. The rate of sedimentation is proportional to r^2. Thus, with increasing particle size, the Brownian motion diminishes while the tendency to sediment increases. The two become equal for a critical radius when the distance h through which the particle settles equals the mean displacement $\bar{x}$ due to Brownian motion in the same time interval t.[35] In most pharmaceutical suspensions, sedimentation prevails. Intravenous vegetable oil emulsions do not tend to cream because the mean droplet size, *ca* 0.5 μm, is smaller than the critical radius.

Passive diffusion caused by a concentration gradient and carried out through Brownian motion is important in the release of drugs from topical preparations (see Chapter 87) and in the gastrointestinal absorption of drugs (see Chapter 35).

Viscosity—Most lyophobic dispersions have viscosities not much greater than that of the liquid vehicle. This holds true even at comparatively high volume fractions of the disperse phase unless the particles form continuous network aggregates throughout the vehicle, in which case yield values are observed. Most O/W and W/O emulsions have specific viscosities not much greater than those predicted by Einstein's modified law of viscosity (see Eq 11 of Chapter 20 and text). For instance, emulsions containing 40% v/v of the internal phase generally have viscosities only three to five times higher than that of the continuous phase. By contrast, the apparent viscosities of lyophilic dispersions, especially of polymer solutions, are several orders of magnitude greater than the viscosity of the solvent or vehicle even at concentrations of only a few percent solids. Lyophilic dispersions are also generally much more pseudoplastic or shear-thinning than lyophobic dispersions (see Chapter 20).

Electric Properties and Stability of Lyophobic Dispersions

Difference between Lyophilic and Lyophobic Dispersions—*Lyophilic* or solvent-loving solids are called hydrophilic if the solvent is water. Owing to the presence of high concentrations of hydrophilic groups, they dissolve or disperse spontaneously in water as far as is possible without breaking covalent bonds. Among hydrophilic groups are ionized ones which dissociate into highly hydrated ions like carboxylate, sulfonate or alkylammonium ions, and organic

functional groups like hydroxyl, carbonyl, amino, and imino which bind water through hydrogen bonding.

The free energy of dissolution or dispersion, ΔG_s, of hydrophilic solids includes a large negative (exothermic) heat or enthalpy of solvation, ΔH_s, and a large increase in entropy, ΔS_s. Since $\Delta G_s = \Delta H_s - T\Delta S_s$, ΔG_s has a large negative value: the dissolution of hydrophilic macromolecules and the dispersion of hydrophilic particulate solids in water occur spontaneously (see Chapter 16), overcoming the parallel increases in surface area and surface free energy. Dissolution and dispersion take place so that water can come into contact and interact with the hydrophilic groups of the solids (enthalpy of solvation), and to increase the number of available configurations of the macromolecules and particles (entropy increase).

The van der Waals energies of attraction between dissolved macromolecules or dispersed hydrophilic solid particles are smaller than ΔG_s and are, therefore, insufficient to cause separation of a solid polymer phase or agglomeration through flocculation or coagulation of the dispersed particles. Furthermore, the hydration layer surrounding dissolved macromolecules and dispersed particles forms a barrier preventing their close approach.

Hydrophobic solids and liquids such as organic compounds consisting largely of hydrocarbon portions with few if any hydrophilic functional groups, like cholesterol and other steroids, and some nonionized inorganic substances like sulfur, are hydrated slightly or not at all. Hence they do not disperse or dissolve spontaneously in water: ΔG_s is positive because of a positive (endothermic) ΔH_s term, making the reverse process (agglomeration) the spontaneous one. Aqueous dispersions of such hydrophobic solids or liquids can be prepared by physical means which supply the appropriate energy to the system (see above). They are unstable, however. The van der Waals attractive forces between the particles cause them to aggregate, since the solvation forces which promote dispersal in water are weak. If aqueous dispersions of hydrophobic solids are to resist reaggregation (coagulation and flocculation), they must be stabilized. Stabilizing factors include electric charges at the particle surface (due to dissociation of ionogenic groups of the solid or pertaining to adsorbed ions such as ionic surfactants) and the presence of adsorbed macromolecules or nonionic surfactants. These stabilizing factors do not alter the intrinsic thermodynamic instability of lyophobic dispersions; ΔG_s is still positive so that the reverse process of phase separation or aggregation is energetically favored over dispersal. They establish kinetic barriers which delay the aggregation processes almost indefinitely; the dispersed particles cannot come together close enough for the van der Waals attractive forces to produce coagulation.[24,26,27] These stabilization mechanisms are discussed below.

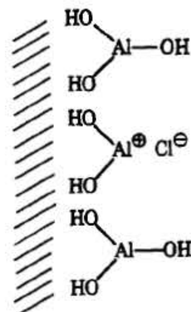
The reductions in surface area and surface free energy accompanying flocculation or coagulation are small because irregular solid particles, being rigid, touch only at a few points upon aggregation. The loose initial contacts may grow with time by sintering or recrystallization. Sintering consists of the "fusion" of primary particles into larger primary particles which propagates from initial small areas of contact. This recrystallization process is spontaneous because it decreases the specific surface area of the disperse solid and the surface free energy of the dispersion. Sintering is analogous to Ostwald ripening, the recrystallization process of transferring solid from colloidal to coarse particles discussed above. Low solubility and the presence of adsorbed surface-active substances retard both processes.

Origin of Electric Charges—Particles can acquire charges from several sources. In *proteins*, one end group of the polypeptide chain and aspartic and glutamic acid units contribute carboxylic acid groups, which are ionized into carboxylate ions in neutral to alkaline media. The other chain end group and lysine units contribute amino groups, arginine units contribute guanidine groups, and histidine units contribute imidazole groups. The nitrogen atoms of these groups become protonated in neutral to acid media. For electroneutrality, these cationic groups require anions, such as Cl^- if hydrochloric acid was used to make the medium acid and to supply the protons. The neutralizing ions, called counterions, dissociate from the ionogenic basic functional groups and can be replaced by other ions of like charge: they are not an integral part of the protein particle but are located in its immediate vicinity. The alkylammonium, guanidinium and imidazolium ions, which are attached to the protein molecule by covalent bonds, confer a positive charge to it. In neutral and alkaline media, Na^+, K^+, Ca^{2+} and Mg^{2+} are among the counterions neutralizing the negative charges of the carboxylate groups. The latter are covalently attached to and constitute an integral part of the protein particle, conferring a negative charge to it.

At an intermediate pH value, which ranges from 4.5 to 7 for the various proteins, the carboxylate anions and the alkylammonium, guanidinium, and imidazolium cations neutralize each other exactly. There is no need for counterions since the ionized functional groups which are an integral part of the protein molecule are in exact balance. At this pH value, called the *isoelectric point*, the protein particle or molecule is neutral; its electric charge is neither negative nor positive, but zero.[22,24,27]

Many other organic polymers contain ionic groups and are, therefore, called *polyelectrolytes* (polymeric electrolytes or salts). Natural polysaccharides of vegetable origin such as acacia, tragacanth, alginic acid and pectin contain carboxylic acid groups, which are ionized in neutral to alkaline media. Agar and carrageenan as well as the animal polysaccharides heparin and chondroitin sulfate, contain sulfuric acid hemiester groups, which are strongly acidic and ionize even in acid media. Cellulosic polyelectrolytes include *sodium carboxymethylcellulose*, while synthetic carboxylated polymers include *carbomer*, a copolymer of acrylic acid.

Aluminum hydroxide, $Al(OH)_3$, is dissolved by acids and alkalis forming aluminum ions, Al^{3+}, and aluminate ions, $[Al(OH)_4]^-$, respectively. In neutral or weakly acid media, at acid concentrations too low to cause dissolution, an aluminum hydroxide particle has some positive charges attributable to incompletely neutralized positive Al^{3+} valences. The portion of the surface of an aluminum hydroxide particle represented schematically below has one such positive charge neutralized by a Cl^- counterion:



In weakly alkaline media, at base concentrations too low to transform the aluminum hydroxide particles completely into aluminate and dissolve them, they bear some negative charges due to the presence of a few aluminate groups. The portion of the particle surface represented schematically below has one such negative group neutralized by a Na^+ counterion:

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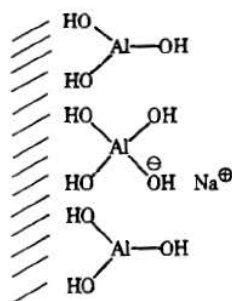
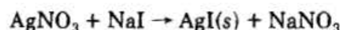


At a pH of 8.5 to 9.1,[36,37] there are neither $[Al(OH)_2]^+$ nor $[Al(OH)_4]^-$ ions in the particle surface but only neutral $Al(OH)_3$ molecules. The particles have zero charge and therefore need no counterions for charge neutralization. This pH is the isoelectric point. In the case of inorganic particulate compounds such as aluminum hydroxide, it is also called zero point of charge.

Bentonite clay is a lamellar aluminum silicate. Each lattice layer consists of a sheet of hydrated alumina sandwiched between two silica sheets. Isomorphous replacement of Al^{3+} by Mg^{2+} or of Si^{4+} by Al^{3+} confers net negative charges to the thin clay lamellas in the form of cation-exchange sites resembling silicate ions built into the lattice. The counterions producing electroneutrality are usually Na^+ (sodium bentonite) or Ca^{2+} (calcium bentonite). The zero point of charge is probably close to that of quartz, silica gel and other silicates, namely, at a pH of about 1.5 to 2.

Silver iodide sols can be prepared by the reaction



$$AgNO_3 + NaI \rightarrow AgI(s) + NaNO_3$$

In the bulk of the silver iodide particles, there is a 1:1 stoichiometric ratio of Ag^+ to I^- ions. If the reaction is carried out with an excess silver nitrate, there will be more Ag^+ than I^- ions in the surface of the particles. The particles will thus be positively charged and the counterions surrounding them will be NO_3^-. If the reaction is carried out using an exact stoichiometric 1:1 ratio of silver nitrate to sodium iodide or with an excess sodium iodide, the surface of the particles will contain an excess I^- over Ag^+ ions.[24,25,27] The particles will be negatively charged, and Na^+ will be the counterions surrounding the particles and neutralizing their charges.

An additional mechanism through which particles acquire electric charges is by the adsorption of ions,[25–27] including ionic surfactants.

Electric Double Layers—The surface layer of a silver iodide particle prepared with an excess of sodium iodide contains more I^- than Ag^+ ions, whereas its bulk contains the two ions in exactly equimolar proportion. The aqueous solution in which this particle is suspended contains relatively high concentrations of Na^+ and NO_3^-, a lower concentration of I^-, and traces of H^+, OH^- and Ag^+.

The negatively charged particle surface attracts positive ions from the solution and repels negative ions: the solution in the vicinity of the surface contains a much higher concentration of Na^+, which are the counterions, and a much lower concentration of NO_3^- ions than the bulk of the solution. A number of Na^+ ions equal to the number of excess I^- ions in the surface (ie, the number of I^- ions in the surface layer minus the number of Ag^+ ions in the surface layer) and equivalent to the net negative surface charge of a particle are pulled towards its surface. These counterions tend to stick to the surface, approaching it as closely as their hydration spheres permit (Helmholtz double layer), but the thermal agitation of the water molecules tends to disperse them throughout the solution. As a result, the layer of counterions surrounding the particle is spread out. The Na^+ concentration is highest in the immediate vicinity of the negative surface, where they form a compact layer called the Stern layer, and decreases with distance from the surface, throughout a diffuse layer called the Gouy-Chapman layer: the sharply defined negatively charged surface is surrounded by a cloud of Na^+ counterions required for electroneutrality. The combination of the two layers of oppositely charged ions constitutes an electric double layer. It is illus-

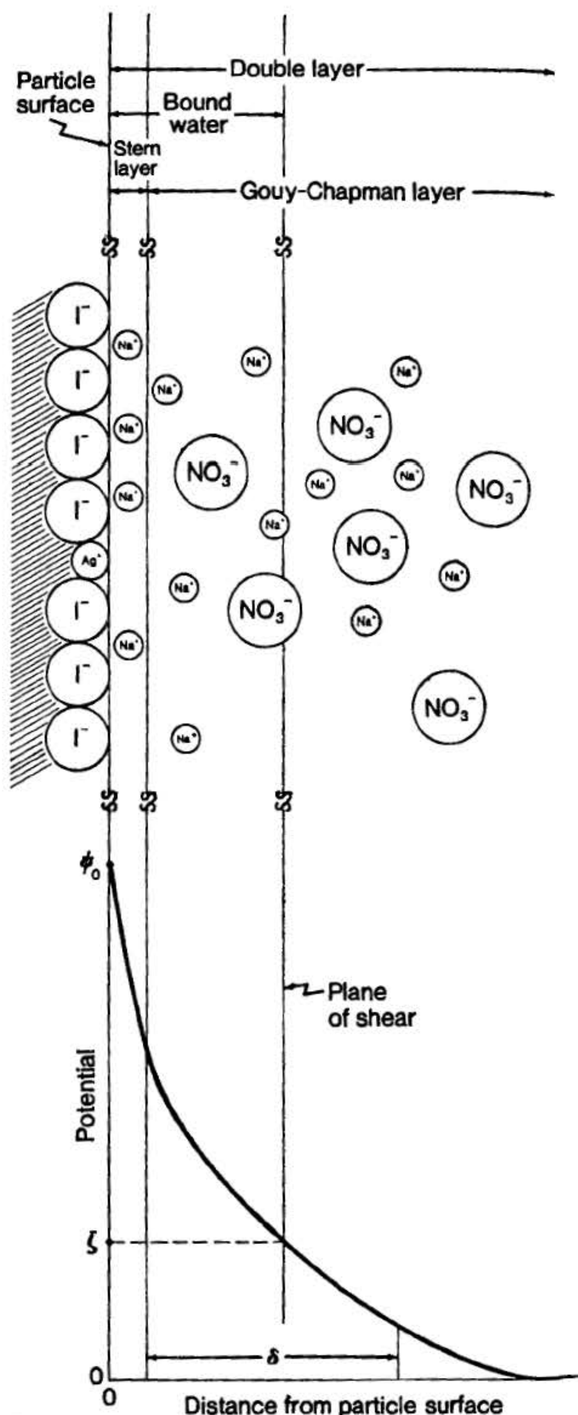


Fig 19-28. Electric double layer at the surface of a silver iodide particle (upper part) and the corresponding potentials (lower part). The distance from the particle surface, plotted on the horizontal axis, refers to both the upper and lower parts.

trated in the top part of Fig 19-28. The horizontal axis represents the distance from the particle surface in both the top and bottom parts.

The electric potential of a plane is equal to the work against electrostatic forces required to bring a unit electric charge from infinity (in this case, from the bulk of the solution) to that plane. If the plane is the surface of the particle, the potential is called surface or ψ_0 potential, which measures the total potential of the double layer. This is the thermodynamic potential which operates in galvanic cells. On moving away from the particle surface towards the bulk solution in the direction of the horizontal axis, the potential drops rapidly across the Stern layer because the Na^+ ions in the immediate vicinity of the surface screen Na^+ ions farther removed, in the diffuse part of the double layer, from the effect of the negative surface charge. The decrease in potential across the Gouy-Chapman layer is more gradual. The diffuse double layer gradually comes to an end as the composition approaches that of the bulk liquid where the anion concentration equals the cation concentration, and the potential approaches zero asymptotically. In view of the indefinite end point, the thickness δ of the diffuse double layer is arbitrarily assigned the value of the distance over which the potential at the boundary between the Stern and Gouy-Chapman layers drops to $1/e = 0.37$ of its value.[24–27] The thickness of double layers usually ranges from 10 to 1000 Å. It decreases as the concentration of electrolytes in solution increases, more rapidly for counterions of higher valence. The value of δ is approximately equal to the reciprocal of the Debye-Hückel theory parameter, κ.

Of practical importance, because it can be measured experimentally, is the electrokinetic or ζ (zeta) potential. In aqueous dispersion, even relatively hydrophobic inorganic particles and organic particles containing polar functional groups are surrounded by a layer of water of hydration attached to them by ion-dipole and dipole-dipole interaction. When a particle moves, this shell of bound water and all ions located inside it move along with the particle. Conversely, if water or a solution flows through a fixed bed of these solid particles, the hydration layer surrounding each particle remains stationary and attached to it. The electric potential at the plane of shear or slip separating the bound water from the free water is the ζ potential. It does not include the Stern layer and only that part of the Gouy-Chapman layer which lies outside the hydration shell. The various potentials are shown on the bottom part of Fig 19-28.

Stabilization by Electrostatic Repulsion—When two uncharged hydrophobic particles are in close proximity, they attract each other by van der Waals secondary valences, mainly by London dispersion forces. For individual atoms and molecules, these forces decrease with the seventh power of the distance between them. In the case of two particles, every atom of one attracts every atom of the other particle. Because the attractive forces are nearly additive, they decay much less rapidly with the interparticle distance as a result of this summation, approximately with the second or third power. Since energies of attraction are equal to force x distance, they decrease approximately with the first or second power of the distance. Therefore, whenever two particles approach each other closely, the attractive forces take over and cause them to adhere. Coagulation occurs as the primary particles aggregate into increasingly larger secondary particles or flocs.

If the dispersion consists of two kinds of particles with positive and negative charges, respectively, the electrostatic attraction between oppositely charged particles is superimposed on the attraction by van der Waals forces, and coagulation is accelerated. If the dispersion contains only one kind, as is customary, all particles have surface charges of the same sign and density. In that case, electrostatic repul-

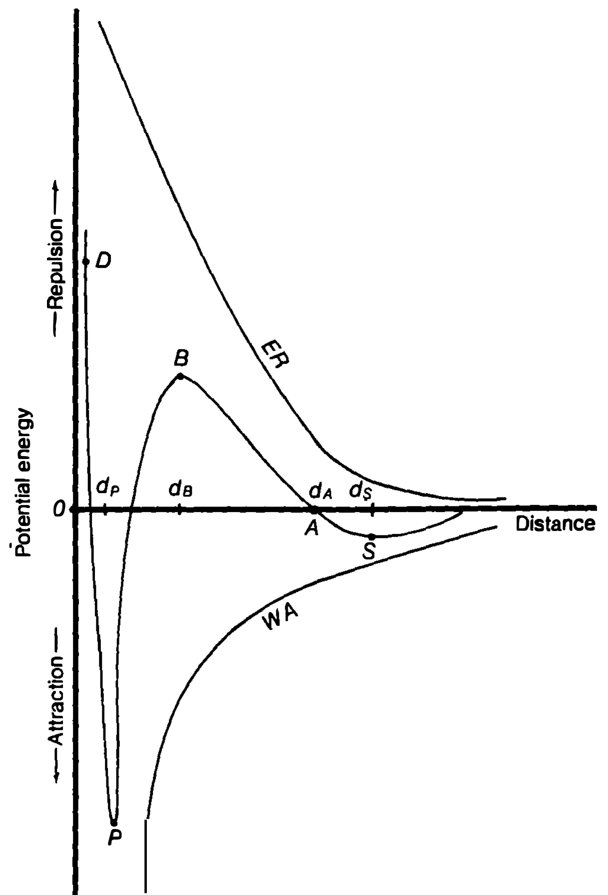


Fig 19-29. Curves representing the van der Waals energy of attraction (*WA*), the energy of electrostatic repulsion (*ER*), and the net energy of interaction (*DPBAS*) between two identical charged particles, as a function of the interparticle distance.

sion tends to prevent the particles from approaching closely enough to come within effective range of each other's van der Waals attractive forces, thus stabilizing the dispersion against interparticle attachments or coagulation. The electrostatic repulsive energy has a range of the order of δ.

A quantitative theory of the interaction between lyophobic disperse particles was worked out independently by Derjaguin and Landau in the USSR and by Verwey and Overbeek in the Netherlands in the early 1940s.[21,24–27,38] Detailed calculations are also found in Chapter 21 of RPS-17. The so-called DLVO theory predicts and explains many but not all experimental data. Its refinement to account for discrepancies is still continuing.

The DLVO theory is summarized in Fig 19-29, where curve *WA* represents the van der Waals attractive energy which decreases approximately with the second power of the interparticle distance, and curve *ER* represents the electrostatic repulsive energy which decreases exponentially with distance. Because of the combination of these two opposing effects, attraction predominates at small and large distances whereas repulsion may predominate at intermediate distances. Negative energy values indicate attraction, and positive values repulsion. The resultant curve *DPBA*, obtained by algebraic addition of curves *WA* and *ER*, gives the total, net energy of interaction between two particles.

The interparticle attraction depends mainly on the chemical nature and particle size of the material to be dispersed. Once these have been selected, the attractive energy is fixed

and cannot readily be altered. The electrostatic repulsion depends on ψ_0 or the density of the surface charge and on the thickness of the double layer, both of which govern the magnitude of the ζ potential. Thus, stability correlates to some extent with this potential.[24] The ζ potential can be adjusted within wide limits by additives, especially ionic surfactants, water-miscible solvents, and electrolytes (see below). If the absolute value of the ζ potential is small, the resultant potential energy is negative and van der Waals attraction predominates over electrostatic repulsion at all distances. Such sols coagulate rapidly.

The two identical particles whose interaction is depicted in Fig 19-29 have a large (positive or negative) ζ potential resulting in an appreciable positive or repulsive potential energy at intermediate distances. They are on a collision course because of Brownian motion, convection currents, sedimentation, or because the dispersion is being stirred.

As the two particles approach each other, the two atmospheres of counterions surrounding them begin to interpenetrate or overlap at point A corresponding to the distance d_A. This produces a net repulsive (positive) energy because of the work involved in distorting the diffuse double layers and in pushing water molecules and counterions aside, which increases if the particles approach further. If the particles continue to approach each other, even after most of the intervening solution of the counterions between them has been displaced, the repulsion between their surface charges increases the net potential energy of interaction to its maximum positive value at B. If the height of the potential energy barrier B exceeds the kinetic energy of the approaching particles, they will not come any closer than the distance d_B but move away from each other. A net positive potential energy of about 25 kT units usually suffices to keep them apart, rendering the dispersion permanently stable; k is the Boltzmann constant and T is the absolute temperature. At $T = 298°K$, this corresponds to 1×10^{-12} erg. The kinetic energy of a particle is of the order of kT.

On the other hand, if their kinetic energy exceeds the potential energy barrier B, the particles continue to approach each other past d_B, where the van der Waals attraction becomes increasingly more important compared to the electrostatic repulsion. Therefore, the net potential energy of interaction decreases to zero and then becomes negative, pulling the particles still closer together. When the particles touch, at a distance d_P, the net energy has acquired the large negative value P. This deep minimum in potential energy corresponds to a very stable situation in which the particles adhere. Since it is unlikely that enough kinetic energy can be supplied to the particles or that their ζ potential can be increased sufficiently to cause them to climb out of the potential energy well P, they are attached permanently to each other. When most or all of the primary particles agglomerate into secondary particles by such a process, the sol coagulates.

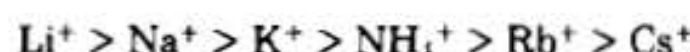
Any closer approach of two particles, than the touching distance d_P, is met with a very rapid rise in potential energy along PD because the solid particles would interpenetrate each other, causing atomic orbitals to overlap (Born repulsion).

Coagulation of Hydrophobic Dispersions—The height of the potential energy barrier and the range over which the electrostatic repulsion is effective (or the thickness of the double layer) determine the stability of hydrophobic dispersions. Both factors are reduced by the addition of electrolytes. The transition between a coagulating and a stable sol is gradual and depends on the time of observation. By using standard conditions, however, it is possible to classify a sol as either coagulated or coagulating, or as stable or fully dispersed.

To determine the value of the coagulating concentration of a given electrolyte for a given sol, a series of test tubes is filled with equal portions of the sol. Identical volumes of solutions of the electrolyte, of increasing concentration, are added with vigorous stirring. After some time at rest (eg, 2 hours), the mixtures are agitated again. After an additional, shorter rest period (eg, ½ hour), they are inspected for signs of coagulation. The tubes can be classified into two groups, one showing no signs of coagulation and the other showing at least some signs, eg, visible flocs. Alternatively, they can be classified into one group showing complete coagulation and the other containing at least some deflocculated colloid left in the supernatant. In either case, the separation between the two classes is quite sharp. The intermediate agitation breaks the weakest interparticle bonds and brings small particles in contact with larger ones, thus increasing the sharpness of separation between coagulation and stability. After repeating the experiment with a narrower range of electrolyte concentrations, the coagulation value c_{CV} of the electrolyte, ie, the lowest concentration at which it coagulates the sol, is established with good reproducibility.[24,25,27]

Typical c_{CV} data for a silver iodide sol prepared with an excess of iodide are listed in Table XIV. The following conclusions can be drawn from the left half of Table XIV:

1. The c_{CV} does not depend on the valence of the anion, since nitrate and sulfate of the same metal have nearly identical values.
2. The differences among the c_{CV}s of cations with the same valence are relatively minor. However, there is a slight but significant trend of decreasing c_{CV} with increasing atomic number in the alkali and in the alkaline earth metal groups. Arranging these cations in the order of decreasing c_{CV} produces the *Hofmeister* or *lyotropic series*. It governs many other colloidal phenomena, including the effect of salts on the temperature of gelation and the swelling of aqueous gels and on the viscosity of hydrosols, the salting out of hydrophilic colloids, the cation exchange on ion-exchange resins, and the permeability of membranes toward salts. The series is also observed in many phenomena involving only small atoms or ions and true solutions, including the ionization potential and electronegativity of metals, the heats of hydration of cations, the size of the hydrated cations, the viscosity, surface tension and infrared spectra of salt solutions, and the solubility of gases therein. For monovalent cations, the lyotropic series is



$$Li^+ > Na^+ > K^+ > NH_4^+ > Rb^+ > Cs^+$$

A similar lyotropic series exists for anions.[21,22,24-26]

The lithium ion has a higher c_{CV} than the cesium ion because it is more extensively hydrated, so that Li^+ (aq), including the hydration shell, is larger than Cs^+ (aq). Owing to its smaller size, the hydrated cesium ion can approach the negative particle surface more closely than the hydrat-

Table XIV—Coagulation Values for Negative Silver Iodide Sol[a]

Electrolyte	c_{CV}, mM/L	Electrolyte	c_{CV}, mM/L
$LiNO_3$	165	$AgNO_3$	0.01
$NaNO_3$	140	½ $(C_{12}H_{25}NH_3)_2SO_4$	0.7
½ Na_2SO_4	141	Strychnine nitrate	1.7
KNO_3	136	½ Morphine sulfate	2.5
½ K_2SO_4	138		
$RbNO_3$	126		
Mean	141		
$Mg(NO_3)_2$	2.60	Quinine sulfate	0.7
$MgSO_4$	2.57		
$Ca(NO_3)_2$	2.40		
$Sr(NO_3)_2$	2.38		
$Ba(NO_3)_2$	2.26		
$Zn(NO_3)_2$	2.50		
$Pb(NO_3)_2$	2.43		
Mean	2.45		
$Al(NO_3)_3$	0.067		
$La(NO_3)_3$	0.069		
$Ce(NO_3)_3$	0.069		
Mean	0.068		

[a] From Ref 21 and unpublished data.

ed lithium ion. Moreover, because of its greater electron cloud, the Cs^+ ion is more polarizable than the Li^+ ion. Therefore, it is more strongly adsorbed in the Stern layer, which makes it a more effective coagulating agent.

3. The coagulation values depend primarily on the valence of the counterions, decreasing by one to two orders of magnitude for each increase of one in their valence (Schulze-Hardy rule). According to the DLVO theory, the coagulation values vary inversely with the sixth power of the valence of the counterions. For mono-, di- and trivalent counterions, they should be in the ratio

$$\frac{1}{1^6} : \frac{1}{2^6} : \frac{1}{3^6} \text{ or } 100 : 1.6 : 0.14$$

The mean c_{CV}'s of Table XIV are 141 : 2.45 : 0.068, or 100 : 1.7 : 0.05, in satisfactory agreement with the DLVO theory.

The following conclusion can be drawn from the right half of Table XIV:

4. The cations on the right side of Table XIV constitute obvious exceptions to the preceding. Ag^+ is the potential-determining counterion. *Potential-determining ions* are those whose concentration determines the surface potential. When silver nitrate is added to the negative silver iodide dispersion, some of its silver ions are incorporated into the negatively charged surface of the particles and lower the magnitude of their charge by reducing the excess of I^- ions in the surface. Thus, silver salts are exceptionally effective coagulating agents because they reduce the magnitude of the ψ_0 as well as of the ζ potential. Indifferent salts, which reduce only the latter, require much higher salt concentrations for comparable reductions in the ζ potential. The other potential-determining ion of silver iodide is I^-. Alkali iodides have higher c_{CV}'s than 141 millimole/liter because they supply iodide ions which enter the surface layer of the silver iodide particles and increase its excess of I^- over Ag^+ ions, thereby making ψ_0 more negative. Bromide and chloride ions act similarly but less effectively.

The principal potential-determining ion for proteins is H^+; those for aluminum hydroxide are OH^- (and hence H^+) and Al^{3+}, but also Fe^{3+} and Cr^{3+} which form mixed hydroxides with Al^{3+}.

5. The cationic surfactant in Table XIV and the alkaloidal salts, which also behave as such, constitute the second exception to the Schulze-Hardy rule. Surface-active compounds contain hydrophilic and hydrophobic moieties in the same molecule, the latter being hydrocarbon portions which by themselves are water-insoluble. Their dual nature causes these compounds to accumulate in interfaces. Dodecylammonium and alkaloidal cations displace inorganic monovalent cations from the Stern layer of a negatively charged silver iodide particle because they are attracted to it not only by electrostatic forces like sodium ions but also by van der Waals forces between their hydrocarbon moieties (dodecyl chains in the case of the dodecylammonium ions) and the solid. Because they are strongly adsorbed from solution onto the surface and do not tend to dissociate from it, surface-active cations are very effective in reducing the ζ potential of the negative silver iodide particles, ie, they have lower c_{CV} than purely inorganic cations of the same valence.

6. Anionic surfactants like those containing lauryl sulfate ions also have a tendency to be adsorbed at solid-liquid interfaces. However, because of electrostatic repulsion between the negatively charged surface of silver iodide particles whose surface layer contains an excess iodide ions and the surface-active anions, adsorption usually does not occur below the critical micelle concentration (see below). If such adsorption does occur, it increases the density of negative charges in the particle surface, raising the c_{CV} of anionic surfactants above that corresponding to their valence.

Ionic solids with surface layers containing the ionic species in near proper stoichiometric balance, and most water-insoluble organic compounds have relatively low surface charge densities. They adsorb ionic surfactants of like charge from solution even at low concentrations, which increases their surface charge densities and the magnitude of their ζ potentials, stabilizing their aqueous dispersions.

The addition of water-miscible solvents such as alcohol, glycerin, propylene glycol or polyethylene glycols to aqueous dispersions lowers the dielectric constant of the medium. This reduces the thickness of the double layer and, therefore, the range over which electrostatic repulsion is effective, and lowers the size of the potential energy barrier. Addition of solvents to aqueous dispersions tends to coagulate them. At concentrations too low to cause coagulation by themselves, solvents make the dispersions more sensitive to coagulation by added electrolytes, ie, they lower the c_{CV}.

Progressive addition of the salt of a counterion of high valence reduces the ζ potential of colloidal particles gradually to zero. Eventually, the sign of the ζ potential may be inverted and its magnitude may increase again, but in the opposite direction. The ψ_0 and ζ potentials of aqueous sulfamerazine suspensions are negative above their isoelectric points; those of bismuth subnitrate are positive. As discussed on page 297, the addition of Al^{3+} to the former and of PO_4^{3-} to the latter in large enough amounts inverts the sign of their ζ potentials; their ψ_0 potentials remain unchanged. Surface-active ions of opposite charge may also produce such charge inversion.

The superposition of the van der Waals attractive energy with its long-range effectiveness and the electrostatic repulsive energy with its intermediate-range effectiveness frequently produces a shallow minimum (designated S in Fig 19-29) in the resultant energy-distance curve at interparticle distances d_S several times greater than δ. If this minimum in potential energy is small compared to kT, Brownian motion prevents aggregation. For large particles such as those of many pharmaceutical suspensions and for particles which are large in one or two dimensions (rods and plates), the *secondary minimum* may be deep enough to trap them at distances d_S from each other. This requires a depth of several kT units. Such fairly long-range and weak attraction produces loose aggregates or flocs which can be dispersed by agitation or by removal or reduction in the concentration of flocculating electrolytes.[21,25–27,38] This reversible aggregation process involving the secondary minimum is called *flocculation*. By contrast, aggregation in the deep primary minimum P, called *coagulation*, is irreversible.

Stabilization by Adsorbed Surfactants—As discussed above, surfactants tend to accumulate at interfaces because of their amphiphilic nature. This process is an *oriented physical adsorption*. Surfactant molecules arrange themselves at the interface between water and an organic solid or liquid of low polarity in such a way that the hydrocarbon chain is in contact with the surface of the solid particle or sticks inside the oil droplet while the polar headgroup is oriented towards the water phase. This orientation removes the hydrophobic hydrocarbon chain from the bulk of the water, where it is unwelcome because it interferes with the hydrogen bonding among the water molecules, while leaving the polar headgroup in contact with water so that it can be hydrated.

Figure 19-30*A* shows schematically that at low surfactant concentration and low surface coverage, the hydrocarbon chains of the adsorbed surfactant molecules lie flat against the solid surface. At higher surfactant concentrations, the surfactant molecules are adsorbed in the upright position to permit the adsorption of more surfactant per unit surface area. Figure 19-30*B* shows a nearly close-packed monolayer of adsorbed surfactant molecules. The terminal methyl groups of their hydrocarbon tails are in contact with the hydrophobic surface and the hydrocarbon tails are in lateral contact with each other. London dispersion forces promote attraction between both types of adjoining groups. The polar headgroups protrude into the water and are hydrated.

The adsorption of ionic surfactants increases the charge density and the ζ potential of the disperse particles. These two parameters are low for organic substances lacking ionic or strongly polar groups. The increase in electrostatic repulsion among the nonpolar organic particles due to adsorption of surface-active ions stabilizes the dispersion against coagulation. This "charge stabilization" is described by the DLVO theory.

Most water-soluble nonionic surfactants are polyoxyethylated (see above): Each molecule consists of a hydrophobic hydrocarbon chain combined with a hydrophilic polyethylene glycol chain, eg $CH_3(CH_2)_{15}(OCH_2CH_2)_{10}OH$. Hydration of the 10 ether groups and of the terminal hydroxyl

group renders the surfactant molecule water-soluble. It adsorbs at the interface between a hydrophobic solid and water, with the hydrocarbon moiety adhering to the solid surface and the polyethylene glycol moiety protruding into the water, where it is hydrated. The particle surface is thus surrounded by a thin layer of hydrated polyethylene glycol chains. This hydrophilic shell forms a steric barrier which prevents close contact between particles and, hence, coagulation ("steric stabilization"). Nonionic surfactants also reduce the sensitivity of hydrophobic dispersions toward coagulation by salts, ie, they increase the coagulation values.[39]

In a flocculated dispersion, groups of several particles are agglomerated into flocs. Frequently, the particles of a floc are in physical contact. When a surfactant is added to a flocculated sol, the dissolved surfactant molecules become adsorbed at the surface of the particles. Surfactant molecules tend to pry apart flocs by wedging themselves between the particles at their areas of contact. This action opens up for surfactant adsorption additional surface area that was previously blocked by adhesion of another solid surface. The breaking up of flocs or secondary particles is defined above as deflocculation or peptization.

Ophthalmic suspensions should be deflocculated because the large particle size of flocs causes eye irritation. Parenteral suspensions should be deflocculated to prevent flocs from blocking capillary blood vessels and hypodermic syringes, and to reduce tissue irritation. Deflocculated suspensions tend to cake, however, ie, the sediment formed by gravitational settling is compact and may be hard to disperse by shaking. Caking in oral suspensions is prevented by controlled flocculation as discussed below.

Stabilization by Adsorbed Polymers—Water-soluble polymers are adsorbed at the interface between water and a hydrophobic solid if they have some hydrophobic groups that limit their water solubility and render them amphiphilic and, hence, surface-active. Such polymers also tend to accumulate at the air-water interface and lower the surface tension of the aqueous phase. A high concentration of ionic groups in polyelectrolytes tends to eliminate surface activity and the tendency to adsorb at interfaces, because the polymer is excessively water-soluble. An example is *sodium carboxymethylcellulose*. *Polyvinyl alcohol* is very water-soluble due to the high concentration of hydroxyl groups and does not adsorb extensively at interfaces. Polyvinyl alcohol is manufactured by the hydrolysis of polyvinyl acetate, which is water-insoluble. Incomplete hydrolysis of, say, only 85% of the acetyl groups produces a copolymer which is water-soluble but surface-active as well. Other surface-active polymers include methylcellulose, hydroxypropyl cellulose, high-molecular-weight polyethylene glycols (polyethylene oxides), and proteins. The surface activity of proteins is due to the presence of hydrophobic groups in the side chains at concentrations too low to cause insolubility in water. Proteins are denatured upon adsorption at air-water and solid-water interfaces.

The long, chain-like polymer molecules are adsorbed from solution onto solid surfaces in the form of loops projecting into the aqueous phase, as shown in Fig 19-31*A*, rather than lying flat against the solid substrate. Only a small portion of the chain segments of an adsorbed macromolecule is actually in contact with and adheres directly to the surface. Because of its great length, however, there are enough of such areas of contact to anchor the adsorbed macromolecule firmly onto the solid. Figure 19-30 is drawn on a much more expanded scale than Fig 19-31.

The sol particles are surrounded by a layer consisting of the adsorbed polymer chains, the water of hydration associated with them, and water trapped mechanically inside the chain loops. This sheath is an integral part of the particle surface. The layers of adsorbed polymer prevent the particles from approaching each other closely enough for the interparticle attraction by London dispersion forces to produce coagulation. These forces are effective only over very small interparticle distances of less than twice the thickness of the adsorbed polymer layer.

The mechanisms of *steric stabilization* by which adsorbed nonionic macromolecules prevent coagulation of hydrophobic sols (*protective action*) are also operative in the stabilization of sols by nonionic surfactants. The difference between adsorbed nonionic surfactants and adsorbed polymers

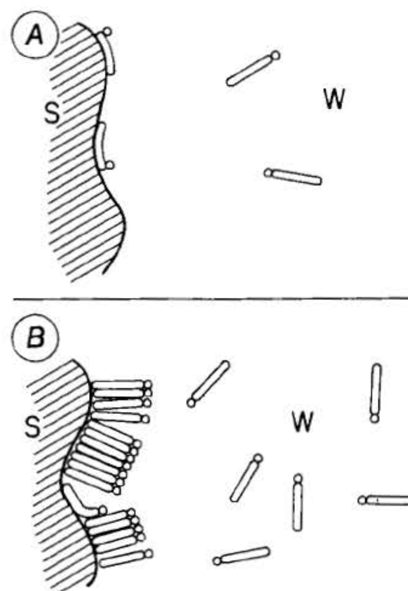


Fig 19-30. Schematic representation of the physical adsorption of surfactant molecules at a hydrophobic solid (S)/water (W) interface. Cylindrical portions and spheres represent hydrocarbon chains and polar headgroups of the surfactant molecules, respectively (*A*) low surfactant concentration/low surface coverage; (*B*) near critical micelle concentration/surface coverage near saturation

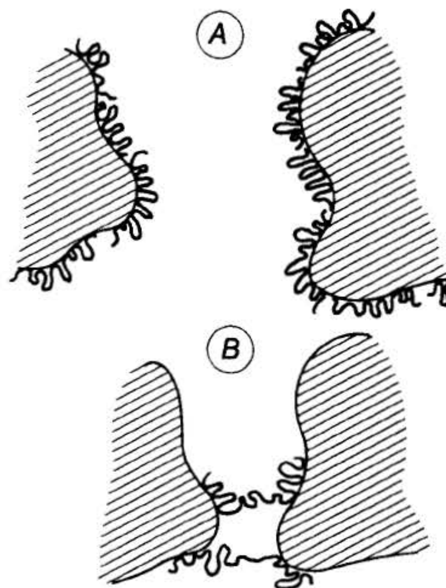


Fig 19-31. Protective action (*A*) and sensitization (*B*) of sols of hydrophobic particles by adsorbed polymer chains.

is that the hydrophilic polyethylene glycol moieties of the adsorbed surfactant molecules protruding into water resemble the chain ends of the adsorbed macromolecules rather than their looped segments. The following protective mechanisms are operative:

1. The layer of adsorbed polymer and enmeshed water surrounding the particles forms a *mechanical* or *steric barrier* between them that prevents the close interparticle approach necessary for coagulation. At dense surface coverage, these layers are somewhat elastic. They may be dented by a collision between two particles but tend to spring back.
2. When two particles approach so closely that their adsorbed polymer layers overlap, the chain loops of the two opposing layers compress and mix with or interpenetrate each other. The resulting restriction to the freedom of motion of the chain segments in the overlap region produces a negative entropy change which tends to make the free energy change for the reduction in interparticle distance required for coagulation positive. The reverse process of disentanglement of the two opposing adsorbed polymer layers resulting from separation of the particles occurs because it is energetically more favorable. The particles are thus prevented from coagulation by *entropic repulsion* through the mechanism of *entropic stabilization* of the sol. This mechanism predominates when the concentration of polymer in the adsorbed layer is low.
3. As the polymer layers adsorbed on two approaching particles overlap and compress or interpenetrate each other, more polymer segments become crowded into a given volume of the aqueous region between the particles. The increased polymer concentration in the overlap region causes a local increase in osmotic pressure, which is relieved by an influx of water. This influx to dilute the polymer loops pushes the two particles apart, preventing coagulation.
4. If the adsorbed polymer has some ionic groups, stabilization by electrostatic repulsion or charge stabilization described above is added to the three steric stabilization mechanisms to prevent a close interparticle approach and, hence, coagulation.
5. The adsorption of water-soluble polymers changes the nature of the surface of the hydrophobic particles to hydrophilic, resulting in an increased resistance of the sol to coagulation by salts.[40]

The water-soluble polymers whose adsorption stabilizes hydrophobic sols and protects them against coagulation are called *protective colloids*. *Gelatin* and *serum albumin* are the preferred protective colloids for stabilizing parenteral suspensions because of their biocompatibility. These two polymers, as well as casein (milk protein), dextrin (partially hydrolyzed starch) and vegetable gums like acacia and tragacanth are metabolized in the human body. Cellulose derivatives and most synthetic protective colloids such as *povidone* are not biotransformed. Because of this and because of their large molecular size, polymers pertaining to the last two categories are not absorbed but excreted intact when they are administered in an oral dosage form.

A semiquantitative assessment of the stabilizing efficiency of protective colloids is the *gold number*, developed by Zsigmondy. It is the largest number of milligrams of a protective colloid which, when added to 10 mL of a special standardized gold sol, just fails to prevent the change in color from red to blue on addition of 1 mL of 10% NaCl solution. The gold sol contains 0.0058% gold with a particle size of about 250 Å. Coagulation by sodium chloride causes the color change. Representative gold numbers are 0.005 to 0.01 for gelatin, 0.01 for casein, 0.02 to 0.5 for egg albumin, 0.15 to 0.5 for acacia, and 1 to 7 for dextrin.[22,27] Gelatin is a more effective protective colloid than acacia or dextrin because the presence of some hydrophobic side groups makes it more surface active and causes more extensive adsorption from solution. Other protective numbers are based on different hydrophobic disperse solids, eg, silver, Prussian blue, sulfur, ferric oxide. The ranking of different protective colloids depends somewhat on the substrate. When formulating a disperse dosage form, one should measure the protective action on the actual solid hydrophobic phase to be dispersed as a sol.

Sensitization is the opposite of protective action, namely, a decrease in the stability of hydrophobic sols. It is brought about by some protective colloids, at concentrations well below those at which they exert a protective action. A protective colloid may, at very low concentrations, flocculate a sol in the absence of added salts and/or lower the coagulation values of the sol.

In the case of nonionic polymers or of polyelectrolytes with charges of the same sign as the sol, flocculation is the result of the bridging mechanism illustrated in Fig 19-31*B*. At very low polymer concentrations, there are not nearly enough polymer molecules present to cover each sol particle completely. Since the particle surfaces are largely bare, a single macromolecule may be adsorbed on two particles, bridging the gap between them and pulling them close together. Flocs of several particles are formed when one particle is bridged or connected to two or more other particles by two or more polymer molecules adsorbed jointly on two or possibly even three particles. Such flocculation usually occurs over a narrow range and at very low values of polymer concentrations. At higher concentrations, when enough polymer is available to cover the surface of all particles completely, bridging is unlikely to occur and the adsorbed polymer stabilizes or peptizes the sol.[23,40]

The nonionic Polymer A of Fig 19-32 stabilizes the sol at all concentrations. Neither sensitization by bridging nor by charge neutralization is observed. The reason that Polymer A lowers the positive ζ potential of the sol slightly is that increasing amounts of adsorbed polymer chains gradually shift the plane of shear outward, away from the positively charged surface. If Polymer A was a cationic polyelectrolyte, the ζ potential–protective colloid concentration plot would gradually rise with increasing polymer adsorption rather than drop.

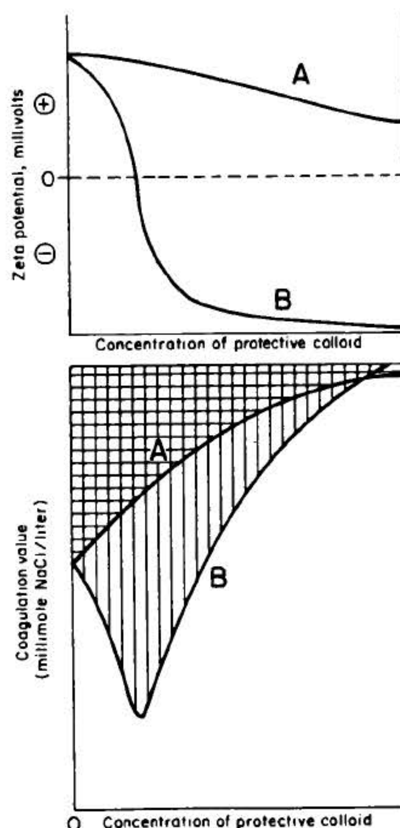


Fig 19-32. Protective action and sensitization: Polymer A exerts protective action at all concentrations, while Polymer B sensitizes at low concentrations and stabilizes at high concentrations. Horizontal and vertical hatching indicates region of flocculation for a sol treated with various concentrations of Polymers A and B, respectively. Clear region underneath indicates sol is deflocculated.

If the polymer has ionic groups of charge opposite to the charge of the sol particles, limited adsorption neutralizes the charge of the particles, reducing their ζ potential to near zero. With stabilization by electrostatic repulsion thus inoperative, and steric stabilization ineffective because of low surface coverage with adsorbed polymer, the sol either coagulates by itself or is coagulated by very small amounts of sodium chloride. At higher polymer concentrations and more extensive adsorption, charge reversal of the particles to the sign of the charge of the polyelectrolyte reactivates charge stabilization and adds steric stabilization, increasing the coagulation value of the sol well above the initial value before polymer addition.

For example, a partly hydrolyzed polyacrylamide with about 20% of ammonium acrylate repeating units is an anionic polyelectrolyte. At the ppm level, the polymer flocculates aluminum hydroxide sols at a pH of 6 to 7, where the sols are positively charged and the polyelectrolyte is fully ionized. At a polymer concentration of 1:10,000, the sol becomes negatively charged because extensive polymer adsorption introduces an excess of $—COO^-$ groups over $=Al^+$ ions into the particle surface. Steric stabilization plus electrostatic repulsion make the sol more stable against flocculation by salts than it was before the polyacrylamide addition.

Polymer B in Fig 19-32 illustrates this example. The curve in the lower plot indicates sensitization, with the coagulation value of sodium chloride lowered by as much as 60%. Zeta potential measurements can distinguish between sensitization by bridging and by charge neutralization. The charge reversal caused by adsorption of Polymer B shown in the upper plot pinpoints charge neutralization as the cause of sensitization. If Polymer B had a ζ potential–polymer concentration plot similar to Polymer A, sensitization would be ascribed to bridging.

Even water-soluble polymers which are too thoroughly hydrophilic to be adsorbed by hydrophobic sol particles can stabilize those sols. Their thickening action slows down Brownian motion and sedimentation, giving the particles less opportunity to come into contact and hence retarding flocculation.

Electrokinetic Phenomena—When a dc electric field is applied to a dispersion, the particles move towards the electrode of charge opposite to that of their surface. The counterions located inside their hydration shell are dragged along while the counterions in the diffuse double layer outside the plane of slip, in the free or mobile solvent, move toward the other electrode. This phenomenon is called *electrophoresis*. If the charged surface is immobile, as is the case with a packed bed of particles or a tube filled with water, application of an electric field causes the counterions in the free water to move towards the opposite electrode, dragging solvent with them. This flow of liquid is called *electroosmosis*, and the pressure produced by it, *electroosmotic pressure*. Conversely, if the liquid is made to flow past charged surfaces by applying hydrostatic pressure, the displacement of the counterions in the free water produces a potential difference between the two ends of the tube or bed called *streaming potential*.

The three phenomena depend on the relative motion of a charged surface and of the diffuse double layer outside the plane of slip surrounding that surface. The major part of the diffuse double layer is within the free solvent and can, therefore, move along the surface.[24–27,41] All three electrokinetic phenomena measure the identical ζ potential, which is the potential at the plane of slip.

The particles of pharmaceutical suspensions and emulsions are visible in the microscope or ultramicroscope, as are bacteria, erythrocytes and other isolated cells, latex particles, and many contaminant particles in pharmaceutical solutions. Their ζ potential is conveniently measured by *microelectrophoresis*. A potential difference E applied between two electrodes dipping into the dispersion and separated by a distance d produces the potential gradient or field strength E/d, expressed in v/cm. From the average velocity v of the particles, measured with the eyepiece micrometer of a microscope and a stopwatch, the ζ potential is calculated by the Smoluchowski equation

$$\zeta = \left(\frac{4\pi\eta}{D}\right)\left(\frac{v}{E/d}\right) = \left(\frac{4\pi\eta}{D}\right)\mu$$

The electrophoretic mobility $u = v/(E/d)$ is the velocity in a potential gradient of 1 v/cm. Particle size and shape do not affect the ζ potential according to the above equation. However, if the particle radius is comparable to δ or smaller (in which case the particles cannot be detected in a microscope), the factor 4 is replaced by 6. The viscosity η and the dielectric constant D refer to the aqueous medium in the double layer and cannot be measured directly.[42] Using the values for water at 25°, expressing the velocity in μm/sec and the electrophoretic mobility in (μm/sec)/(volts/cm), and converting into the appropriate units reduces the Smoluchowski equation to $\zeta = 12.9\,u$, with ζ given in millivolts (mV). If the particle surface has appreciable conductance, the ζ potential calculated by this equation may be low.[25,41,42] Dispersions of hydrophobic particles with ζ potentials below 20–30 mV are frequently unstable and tend to coagulate. On the other hand, values as high as ±180 mV have been reported for the ζ potential.[21,24,41]

The chief experimental precautions in microelectrophoresis measurements are:

1. Electroosmosis causes liquid to flow along the walls of the cell containing the dispersion. This in turn produces a return flow in the center of the cell. The microscope must be focused on the stationary boundary between the two liquid layers flowing in opposite directions in order to measure the true velocity of the particles.
2. Only in very dilute dispersions is it possible to follow the motion of single particles in the microscope field and to measure their velocity. Since the ζ potential depends largely on the nature, ionic strength, and pH of the suspending medium, dispersions should be diluted not with water but with solutions of composition identical to their continuous phase, eg, with their own serum separated by ultrafiltration or centrifugation. The Zeta-Meter is a commercial microelectrophoresis apparatus of easy, fast and reproducible operation.

When the particles cannot be observed individually with a microscope or ultramicroscope, other electrophoresis methods are employed.[24,27,41,43,44] In *moving boundary electrophoresis*, the movement of the boundary formed between a sol or solution and the pure dispersion medium in an electric field is studied. If the disperse phase is colorless, the boundary is located by the refractive index gradient (Tiselius apparatus, used frequently with protein solutions). If several species of particles or solutes with different mobilities are present, each will form a boundary moving with a characteristic velocity. Unlike microelectrophoresis, this method permits the identification of different colloidal components in a mixture, the measurement of the electrophoretic mobility of each, and an estimation of the relative amounts present.

Zone electrophoresis theoretically permits the complete separation of all electrophoretically different components, requires much smaller samples than moving boundary electrophoresis, and can be performed in simpler and less expensive equipment. The method avoids convection by supporting the solution in an inert and porous solid like filter paper, cellulose acetate membrane, agar, starch or polyacrylamide gels cut into strips, or disks or columns of polyacrylamide gel.

A strip of filter paper or gel is saturated with a conducting buffer solution and a few microliters of the solution being analyzed is deposited as a spot or narrow band. A potential difference is applied between the ends of the strip which are

in contact with the electrode compartments. The spot or band spreads and unfolds as each component migrates towards one or the other electrode at a rate determined primarily by its electrophoretic mobility. Evaporation of water due to the heating effect of the electric current may be minimized by immersing the strip in a cooling liquid or sandwiching it between impervious solid sheets. After a sufficient time has elapsed to afford good separation, the strip is removed and dried. The position of the spots or bands corresponding to the individual components is detected by color reactions or radioactive counting.

Zone electrophoresis is applied mainly in analysis and for small-scale preparative separations. It does not permit mobility measurements. Because several samples can be analyzed simultaneously (in parallel strips or gel columns), because only minute amounts of sample are needed, and because the equipment is simple and easy to operate, zone electrophoresis is widely used to study the proteins in blood serum, erythrocytes, lymph and cerebrospinal fluid, saliva, gastric and pancreatic juices and bile.

Immunodiffusion combined with electrophoresis is called *immunoelectrophoresis*.[43,45] The proteins in a fluid, including the antigens, are first separated by gel electrophoresis. A longitudinal trench is then cut along one or both sides of the gel strip near the edge in the direction of the electrophoresis axis. The trench is filled with the antibody solution. On standing, antibody and antigen proteins diffuse in all directions, including toward each other. Precipitation occurs along an elliptical arc (precipitin band) wherever an antigen meets its specific antibody. The precipitin bands are either visible directly or may be developed by staining. Since diseases frequently produce abnormal electrophoretic patterns in body fluids, zone electrophoresis and immunoelectrophoresis are convenient and powerful diagnostic techniques.

Isoelectric focusing[44,46] uses electrophoresis to separate proteins according to their isoelectric points. At pH values equal to their isoelectric points, proteins do not migrate in an electric field because their net charge is zero. In a liquid column on which a pH gradient is imposed, different species arrange themselves so that the protein with the highest isoelectric point will be located nearest to the cathode, which is immersed in the solution of a strong base. The protein with the lowest isoelectric point will be located nearest to the anode, which is immersed in the solution of a strong acid. The other proteins settle into intermediate positions, where the pH values are intermediate and equal to their isoelectric points.

Hydrophilic Dispersions

Most liquid disperse systems of pharmaceutical interest are aqueous. Therefore, most lyophilic colloidal systems discussed below consist of hydrophilic solids dissolved or dispersed in water. Most of the products mentioned below are official in the USP or NF, where more detailed descriptions may be found, also elsewhere in this text.

Hydrophilic colloids can be divided into particulate and soluble materials. The latter are water-soluble linear or branched polymers dissolved molecularly in water. Their aqueous solutions are classified as colloidal dispersions because the individual molecules are in the colloidal particle size range, exceeding 50 or 100 Å. Particulate or corpuscular hydrophilic colloidal dispersions are formed by solids which swell and are peptized in water but whose primary particles do not dissolve or break down into individual molecules or ions. One subdivision of particulate hydrophilic colloids is comprised of dispersions of cross-linked polymers whose linear, uncross-linked analogues are water-soluble.

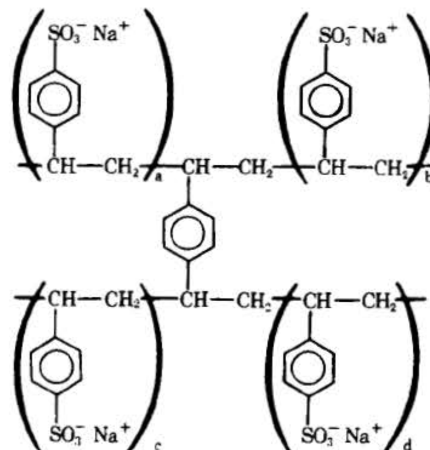
Particulate Hydrophilic Dispersions

The disperse phase of these sols consists of solids which in water swell and break up spontaneously into particles of colloidal dimensions. The disperse particles have high specific surface areas and are, therefore, extensively hydrated. They have characteristic shapes. If the attraction between individual particles is strong, the dispersions have yield values at relatively low solids content.

Bentonite is an aluminum silicate crystallizing in a layer structure (see above), with individual lamellas 9.4 Å thick. Their top and bottom surfaces are sheets of oxygen ions from silica plus an occasional sodium ion neutralizing a silicate ion-exchange site. The clay particles consist of stacks of these lamellas. Water penetrates inside the stacks between lamellas to hydrate the oxygen ions, causing extensive swelling. Bentonite particles in bentonite magma consist of single lamellas and packets of a few lamellas with intercalated water. The specific surface area amounts to several hundred square meters per gram. *Kaolin* also has a layer structure, but does not swell in water because water does not intercalate between individual lattice layers. Kaolin plates dispersed in water are, therefore, much thicker than those of bentonite, *ca* 0.04 to 0.2 μm. In kaolin, hydrated alumina lattice planes alternate with silica planes. Thus, one of the two external surfaces of a kaolin plate consists of a sheet of oxygen ions from silica, the other is a sheet of hydroxide ions from hydrated alumina. Both surfaces are well hydrated. Magnesium aluminum silicate (*Veegum*) is a clay similar to bentonite but contains magnesium; it is white whereas bentonite is gray.

Additional hydrophilic particles producing colloidal dispersions in water are listed below. *Colloidal silicon dioxide* consists of roughly spherical particles covered with siloxane and silanol groups (pages 280–281). *Titanium dioxide* is a white pigment with excellent covering power due to its high refractive index. *Microcrystalline cellulose* (page 279) is hydrophilic because of the hydroxyl and ether groups in the surface of the cellulose crystals. Gelatinous precipitates of hydrophilic compounds such as *aluminum hydroxide gel*, *aluminum phosphate gel*, and *magnesium hydroxide* consist of coarse flocs produced by agglomeration of the colloidal particles formed in the initial stage of the precipitation. They possess large internal surface areas, which is one of the reasons why the first two are used as substrates for adsorbed vaccines and toxoids.

Cross-linked Polymers—The polymers discussed below are polyelectrolytes, ie, they contain ionic groups and would be soluble in water in the absence of cross-linking. For instance, *sodium polystyrene sulfonate* is a copolymer of about 92% styrene and 8% divinylbenzene, which is sulfonated and neutralized to produce the cation-exchange resin



Chains a–b and c–d are water-soluble linear polymer chains. They are cross-linked or bound together via a phenylene group as shown. There are many such cross-links tieing every chain to two or more other chains, so that every atom in a grain of ion-exchange resin is bound to every other atom by primary, covalent bonds. The grains swell in water until the cross-links are strained but do not dissolve, because this would involve the rupture of primary valence bonds. Swelling renders the ion-exchange sites in the interior of a grain accessible to the gastrointestinal fluids. Partial exchange of Na^+ by K^+ followed by excretion of the used resin in the feces reduces hyperkalemia resulting from acute renal failure. Partial replacement of Na^+ by H^+ could reduce acidosis.

Cholestyramine resin is an anion-exchange resin containing the same backbone of cross-linked polystyrene, but substituted with $—CH_2—N^+(CH_3)_3Cl^-$ instead of sodium sulfonate. Part of the chloride anions is exchanged or replaced by bile salt anions, which are thus eliminated in the feces bound to the resin grains rather than reabsorbed. *Colestipol hydrochloride* is another orally administered anion-exchange resin used to increase the fecal excretion of bile salts. It is an extensively cross-linked, insoluble but permeable copolymer made from diethylenetriamine, tetraethylenepentamine, and epichlorohydrin. Strong cation- and anion-exchange resins are used as sustained-release vehicles for basic and acid drugs, respectively (see Chapter 91).

Polycarbophil is a copolymer of acrylic acid cross-linked with a small amount of divinyl glycol. The weakly acidic carboxyl groups are not ionized in the strongly acid environment of the stomach but only in the more nearly neutral intestines. Therefore, swelling by osmotic influx of water occurs mostly in the intestines, where imbibition of water decreases the fluidity of stools associated with diarrhea. Among natural polymers, tragacanth consists of ⅓ of a water-soluble fraction, tragacanthin, and ⅔ of a gel fraction called bassorin which swells in water but does not dissolve. Starch consists of ⅙ of a fraction, soluble in hot water, called amylose. The remainder, amylopectin, merely absorbs water and swells. It owes its insolubility to extensive branching rather than cross-linking.

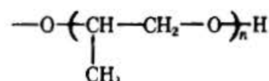
Soluble Polymers as Lyophilic Colloids

Most hydrophilic colloidal systems used in dosage forms are molecular solutions of water soluble, high molecular weight polymers. The polymers are either linear or slightly branched but not cross-linked.

Classifications—According to their origin, water-soluble polymers are divided into three classes. *Natural polymers* include polysaccharides (acacia, agar, heparin sodium, pectin, sodium alginate, tragacanth, xanthan gum) and polypeptides (casein, gelatin, protamine sulfate). Of these, agar and gelatin are only soluble in hot water.

Cellulose derivatives are produced by chemical modification of cellulose obtained from wood pulp or cotton to produce soluble polymers. *Cellulose* is an insoluble, linear polymer of glucose repeat units in the ring or pyranose form joined by β-1,4 glucosidic linkages. Each glucose repeat unit (except for the two terminal ones) contains a primary hydroxyl group on the No 6 carbon and two secondary hydroxyls on No 2 and 3 carbons. The primary hydroxyl is more reactive. Chemical modification of cellulose consists in reactions or substitutions of the hydroxyl groups. The extent of such reactions is expressed as *degree of substitution* (DS), namely, the number of substituted hydroxyl groups per glucose residue. The highest value is DS = 3.0. Fractional values are the rule because the DS is averaged over a multitude of glucose residues. A DS value of 0.6 indicates that some glucose repeat units are unsubstituted while others have one or even two substituents.

Soluble cellulose derivatives are listed below. The DS values correspond to the pharmaceutical grades. The groups shown are the replacements for the hydrogen atoms of the cellulosic hydroxyls. Official derivatives are *methylcellulose* (DS = 1.65–1.93), $—O—CH_3$ and *sodium carboxymethylcellulose* (DS = 0.60–1.00), $—O—CH_2—COO^-Na^+$. *Hydroxyethyl cellulose* (DS ≅ 1.0), $—O(CH_2CH_2—O)_nH$ and *hydroxypropyl cellulose* (DS ≅ 2.5) are manufactured



by the addition of ethylene oxide and propylene oxide, respectively, to alkali-treated cellulose. The value of n is about 2.0 for the former and not much greater than 1.0 for the latter. *Hydroxypropyl methylcellulose* is prepared by reacting alkali-treated cellulose first with methyl chloride to introduce methoxy groups (DS = 1.1–1.8) and then with propylene oxide to introduce propylene glycol ether groups (DS = 0.1–0.3). In general, the introduction of hydroxypropyl groups into cellulose reduces the water solubility somewhat while promoting the solubility in polar organic solvents like short-chain alcohols, glycols and some ethers.

The molecular weight of native cellulose is so high that soluble derivatives of approximately the same degree of polymerization would dissolve too slowly, and their solutions would be excessively viscous even at concentrations of 1% and less. Controlled degradation is used to break the cellulose chains into shorter segments, reducing the viscosity of the solutions of the corresponding soluble derivatives. Commercial grades of a given cellulose derivative such as sodium carboxymethylcellulose come in various molecular weights or viscosity grades as well as with various degrees of substitution, offering the pharmacist a wide selection.

Official cellulose derivatives which are insoluble in water but soluble in some organic solvents include *ethylcellulose* (DS = 2.2–2.7), $—O—C_2H_5$; *cellulose acetate phthalate* (DS = 1.70 for acetyl and 0.77 for phthalyl); and *pyroxylin* or cellulose nitrate (DS ≅ 2), $—O—NO_2$. *Collodion*, a 4.0% w/v solution of pyroxylin in a mixture of 75% (v/v) ether and 25% (v/v) ethyl alcohol, constitutes a lyophilic colloidal system.

The third class, water soluble *synthetic polymers*, consists mostly of vinyl derivatives including *polyvinyl alcohol, povidone* or polyvinylpyrrolidone, and *carbomer* (*Carbopol*), a copolymer of acrylic acid. High molecular weight polyethylene glycols are also called *polyethylene oxides*.

A second classification of hydrophilic polymers is based on their charge. *Nonionic* or uncharged polymers include methylcellulose, hydroxyethyl and hydroxypropyl cellulose, ethylcellulose, pyroxylin, polyethylene oxide, polyvinyl alcohol and povidone. *Anionic* or negatively charged *polyelectrolytes* include the following carboxylated polymers: acacia, alginic acid, pectin, tragacanth, xanthan gum and carbomer at pH values leading to ionization of the carboxyl groups; sodium alginate and sodium carboxymethylcellulose; also polypeptides at pH values above their isoelectric points, eg, sodium caseinate. A stronger acid group is sulfuric acid, which exists as a monoester in agar and heparin and as a monoamide in heparin. *Cationic* or positively charged *polyelectrolytes* are rare. Examples are polypeptides at pH values below their isoelectric points. Protamines are strongly basic due to a high arginine content, with isoelectric points around pH 12, eg protamine sulfate.

Gel Formation—As described in Chapter 20 and illustrated in Fig 20-7*A*, the flexible chains of dissolved polymers interpenetrate and are entangled because of the constant Brownian motion of their segments. The chains writhe and forever change their conformations. Each chain is encased in a sheath of solvent molecules that solvate its functional groups. In the case of aqueous solutions, water molecules

are hydrogen-bonded to the hydroxyl groups of polyvinyl alcohol, hydroxyl groups and ether links of polysaccharides, ether links of polyethylene oxide or polyethylene glycol, amide groups of polypeptides and povidone, and carboxylate groups of anionic polyelectrolytes. The envelope of water of hydration prevents chains segments in close proximity from touching and attracting one another by interchain hydrogen bonds and van der Waals forces as they do in the solid state. The slippage of solvated chains past one another when the solution flows is lubricated by the free solvent between their solvation sheaths.

Factors that lower the hydration of dissolved macromolecules reduce or thin out the sheath of hydration separating adjacent chains. When the hydration is low, contiguous chains tend to attract one another by secondary valence forces including hydrogen bonds and van der Waals forces. Hydrophobic bonding makes an important contribution to interchain attraction between polypeptide chains even in solution. Van der Waals forces and hydrogen bonds thus establish weak and reversible cross-links between chains at their points of contact or entanglement, bringing about phase separation or precipitation.

Most water-soluble polymers have higher solubilities in hot than in cold water and tend to precipitate on cooling, as the sheaths of hydration surrounding adjacent chains become too sparse to prevent interchain attraction. Dilute solutions separate into a solvent phase practically free of polymer and a viscous liquid phase containing practically all of the polymer but still a large excess of solvent. This process is called *simple coacervation* and the polymer-rich liquid phase a *coacervate*.[21,47] If the polymer solution is concentrated enough and/or the temperature low enough, cooling causes the formation of a continuous network of precipitating chains attached to one another through weak cross-links consisting of interchain hydrogen bonds and van der Waals forces at the points of mutual contact. Segments of regularly sequenced polymer chains even associate laterally into crystalline bundles or crystallites. Irregular chain structures as found in random copolymers, randomly substituted cellulose ethers and esters, and highly branched polymers like acacia prevent crystallization during precipitation from solution. Chain entanglements provide the sole temporary cross-links in those cases. The network of associated polymer chains immobilizes the solvent and causes the solution to set to a gel. Gelatinous precipitates or highly swollen flocs may separate when cooling more dilute polymer solutions.

Besides the chemical nature of polymer and solvent, the three most important factors causing phase separation, precipitation and gelation of polymer solutions are temperature, concentration and molecular weight. Lower temperatures, higher concentrations and higher molecular weights promote gelation and produce stronger gels.

For a typical *gelatin*, 10% solutions acquire yield values and begin to gel at about 25°, 20% solutions at about 30° and 30% solutions at about 32°. The *gelation* is reversible: the gels liquefy when heated above these temperatures. Gelation is rarely observed above 34° regardless of concentration, so that gelatin solutions do not gel at 37°. Conversely, gelatin will dissolve readily in water at body temperature. The gelation temperature or gel point of gelatin is highest at the isoelectric point, where the attachment between adjacent chains by coulombic attraction or ionic bonds between carboxylate ions and alkylammonium, guanidinium or imidazolium groups is most extensive. Since the carboxyl groups are not ionized at gastric pH, interchain ionic bonds are practically nonexistent, and interchain attraction is limited to hydrogen bonds and van der Waals forces. The gelation temperature or the melting point of gelatin gels depends more strongly on temperature and concentration than on pH.[48,49] The combination of an acid pH considerably below the isoelectric point and a temperature of 37° completely prevents the gelation of gelatin solutions. Conversely, these two conditions promote rapid dissolution of gelatin capsules in the stomach. Agar and pectic acid solutions set to gels at only a few percent of solids.

Unlike most water-soluble polymers, methylcellulose, hydroxypropyl cellulose and polyethylene oxide are more soluble in cold than in hot water. Their solutions therefore tend to gel on heating (*thermal gelation*).

When dissolving powdered polymers in water, temporary gel formation often slows the process down considerably. As water diffuses into loose clumps of powder, their exterior frequently turns to a cohesive gel of solvated particles encasing dry powder. Such blobs of gel dissolve very slowly because of their high viscosity and the low diffusion coefficient of the macromolecules. Especially for large-scale dissolution, it is helpful to disperse the polymer powder in water before it can agglomerate into lumps of gel. In order to permit dispersion to precede hydration and to prevent temporary gel formation, the polymer powders are dispersed in water at temperatures where the solubility of the polymer is lowest. Most polymer powders, such as sodium carboxymethylcellulose, are dispersed with high shear in *cold* water before the particles can hydrate and swell to sticky gel grains agglomerating into lumps. Once the powder is well dispersed, the solution is heated with moderate shear to about 60° for fastest dissolution. Because methylcellulose hydrates most slowly in hot water, the powder is dispersed with high shear in ⅕ to ⅓ of the required amount of water heated to 80 to 90°. Once the powder is finely dispersed, the rest of the water is added cold or even as ice, and moderate stirring causes prompt dissolution. For maximum clarity, fullest hydration and highest viscosity, the solution should be cooled to 0 to 10° for about an hour.

The following are two alternative methods for preventing the formation of gelatinous lumps upon addition of water. The powder is prewetted with a water-miscible organic solvent such as ethyl alcohol or propylene glycol that does not swell the polymer, in the proportion of from three to five parts solvent to each part of polymer. If other nonpolymeric powdered adjuvants are to be incorporated into the solution, these are dry-blended with the polymer powder. The latter should comprise ¼ or less of the blend for best results.

A pharmaceutical application of *gelation* in a nonaqueous medium is the manufacture of *Plastibase* or *Jelene* (*Squibb*), which consists of 5% of a low-molecular-weight polyethylene and 95% of mineral oil. The polymer is soluble in mineral oil above 90°, which is close to its melting point. When the solution is cooled below 90°, the polymer precipitates and causes gelation. The mineral oil is immobilized in the network of entangled, and adhering, insoluble polyethylene chains which probably even associate into small crystalline regions. Unlike petrolatum, this gel can be heated to about 60° without substantial loss in consistency.

Large increases in the concentration of polymer solutions may lead to precipitation and gelation. One way of effectively increasing the concentration of aqueous polymer solutions is to add inorganic salts. The salts will bind part of the water of the polymer solution in order to become hydrated. Competition for water of hydration dehydrates the polymer molecules and precipitates them, causing gelation. This phenomenon is called *salting out*. Because of its high solubility in water, ammonium sulfate is often used by biochemists to precipitate and separate proteins from dilute solution. To the pharmacist, salting out usually represents an undesirable problem. It is reversible, however, and subsequent addition of water redissolves the precipitated polymers and liquefies their gels. Salting out may cause the polymer to separate as a concentrated and viscous liquid solution or simple coacervate rather than as a solid gel.

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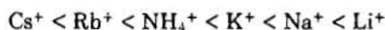
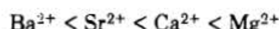
The effectiveness of electrolytes to salt out, precipitate or gel hydrophilic colloidal systems depends on how extensively the electrolytes are hydrated. The *Hofmeister* or *lyotropic series* arranges ions in the order of increasing hydration and increasing effectiveness in salting out hydrophilic colloids. The series, for monovalent cations, is



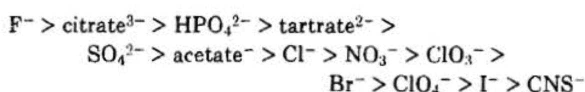
$$Cs^+ < Rb^+ < NH_4^+ < K^+ < Na^+ < Li^+$$

and for divalent cations,



$$Ba^{2+} < Sr^{2+} < Ca^{2+} < Mg^{2+}$$

This series also arranges the cations in the order of decreasing coagulating power or increasing coagulation values for negative hydrophobic sols (see Table XIV) and of increasing ease of their displacement from cation exchange resins: K^+ displaces Na^+ and Li^+. For anions, the lyotropic series in the order of decreasing coagulating power and decreasing effectiveness in salting out is



$$F^- > \text{citrate}^{3-} > HPO_4^{2-} > \text{tartrate}^{2-} > SO_4^{2-} > \text{acetate}^- > Cl^- > NO_3^- > ClO_3^- > Br^- > ClO_4^- > I^- > CNS^-$$

Iodides and thiocyanates and to a lesser extent bromides and nitrates actually tend to increase the solubility of polymers in water, salting them in.[21,22,24-26] These large polarizable anions destructure water, reducing the extent of hydrogen bonding among water molecules and thereby making more of the hydrogen-bonding capacity of water available to the solute. Most salts except nitrates, bromides, perchlorates, iodides and thiocyanates raise the temperature of precipitation or gelation of most hydrophilic colloidal solutions or their gel melting points. Exceptions among hydrophilic colloids are methylcellulose, hydroxypropyl cellulose and polyethylene oxide whose gelation temperatures or gel points and gel melting points are lowered by salting out.

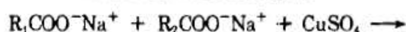
Hydrophobic aqueous dispersions are coagulated by electrolytes at 0.0001–0.1 *M* concentrations (see Table XIV). Moreover, the coagulation is irreversible, ie, removal of the coagulating salt does not allow the coagulum to be redispersed, because the hydrophobic sols are intrinsically unstable. By contrast, most hydrophilic sols require electrolyte concentrations of 1 *M* or higher for precipitation. Their precipitation or gelation can be reversed, and the polymer redissolved by removing the salt through dialysis or by adding more water. Hydrophilic colloids disperse or dissolve spontaneously in water, and their sols are intrinsically stable.

Most of the hydrophilic and water-soluble polymers mentioned above are only slightly soluble or insoluble in alcohol. Addition of alcohol to their aqueous solutions may cause precipitation or gelation because alcohol is a nonsolvent or precipitant, lowering the dielectric constant of the medium, and it tends to dehydrate the hydrophilic solute. Alcohol lowers the concentrations at which electrolytes salt out hydrophilic colloids. Phase separation through the addition of alcohol to an aqueous polymer solution may cause coacervation, ie, the separation of a concentrated viscous liquid phase, rather than precipitation or formation of a gel. Sucrose also competes for water of hydration with hydrophilic colloids, and may cause phase separation. However, most hydrophilic sols tolerate substantially higher concentrations of sucrose than of electrolytes or alcohol. Lower viscosity grades of a given polymer are usually more resistant to electrolytes, alcohol and sucrose than grades of higher viscosity and higher molecular weights.

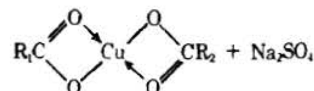
Whenever hydrophilic colloidal dispersions undergo irreversible precipitation or gelation, chemical reactions are involved. Neither dilution with water nor heating nor attempts to remove the gelling or precipitating agent by washing or dialysis will liquefy those gels or redissolve the gelatinous precipitates formed at lower polymer concentrations. Carboxyl groups are not ionized in strongly acid media. If a polymer owes its solubility to the ionization of these weakly acid groups, reducing the pH of its solution below 3 may lead to precipitation or gelation. This is observed with such carboxylated polymers as many gums, sodium carboxymethylcellulose and carbomer. Hydrogen carboxymethylcellulose swells and disperses but does not dissolve in water. Neutralization to higher pH values returns the carboxyl groups to their ionized state and reverses the gelation or precipitation.

Only the sodium, potassium, ammonium and triethanolammonium salts of carboxylated polymers are well soluble in water. In the case of carboxymethylcellulose, salts with heavy metal cations (silver, copper, mercury, lead) and trivalent cations (aluminum, chromic, ferric) are practically insoluble. Salts with divalent cations, especially of the alkaline earth metals, have borderline solubilities. Generally, higher degrees of substitution tend to increase the tolerance of the carboxymethylcellulose to salts.

Precipitation or gelation occur due to metathesis when inorganic salts of heavy or trivalent cations are mixed with alkali metal salts of carboxylated polymers in solution. For instance, if a soluble copper salt is added to a solution of sodium carboxymethylcellulose, the double decomposition can be written schematically as



$$R_1COO^-Na^+ + R_2COO^-Na^+ + CuSO_4 \longrightarrow$$



R_1 and R_2 represent two carboxymethylcellulose chains which are cross-linked by a chelated copper ion. Dissociation of the cupric carboxylate complex is negligible.

Particle Phenomena and Coarse Dispersions

The Dispersion Step

The pharmaceutical formulator is concerned primarily with producing a smooth, uniform, easily flowing (pouring or spreading) suspension or emulsion in which dispersion of particles can be effected with minimum expenditure of energy.

In preparing suspensions, particle–particle attractive forces need to be overcome by the high shearing action of such devices as the colloid mill, or by use of surface-active agents. The latter greatly facilitate wetting of lyophobic powders and assist in the removal of surface air that shearing alone may not remove; thus the clumping tendency of the particles is reduced. Moreover, lowering of the surface free energy by the adsorption of these agents directly reduces the thermodynamic driving force opposing dispersion of the particles.

In emulsification shear rates are frequently necessary for dispersion of the internal phase into fine droplets. The shear forces are opposed by forces operating to resist distortion and subsequent breakup of the droplets. Again surface-active agents help greatly by lowering interfacial ten-

sion, which is the primary reversible component resisting droplet distortion. Surface-active agents also may play an important role in determining whether an oil-in-water or a water-in-oil emulsion preferentially survives the shearing action.

Once the process of dispersion begins there develops simultaneously a tendency for the system to revert to an energetically more stable state, manifested by flocculation, coalescence, sedimentation, crystal growth, and caking phenomena. If these physical changes are not inhibited or controlled, successful dispersions will not be achieved or will be lost during shelf life.

Settling and Its Control

In order to control the settling of dispersed material in suspension, the pharmacist must be aware of those physical factors that will affect the rate of sedimentation of particles under ideal and nonideal conditions. He must also be aware of the various coefficients used to express the amount of flocculation in the system and the effect flocculation will have on the structure and volume of the sediment.

Sedimentation Rate

The rate at which particles in a suspension sediment is related to their size and density and the viscosity of the suspension medium. Brownian movement may exert a significant effect, as will the absence or presence of flocculation in the system.

Stokes' Law—The velocity of sedimentation of a uniform collection of spherical particles is governed by Stokes' law, expressed as follows:

$$v = \frac{2r^2(\rho_1 - \rho_2)g}{9\eta} \quad (35)$$

where v is the terminal velocity in cm/sec, r is the radius of the particles in cm, ρ_1 and ρ_2 are the densities (g/cm^3) of the dispersed phase and the dispersion medium, respectively, g is the acceleration due to gravity (980.7 cm/sec^2) and η is the Newtonian viscosity of the dispersion medium in poises (g/cm sec). Stokes' law holds only if the downward motion of the particles is not sufficiently rapid to cause turbulence. Micelles and small phospholipid vesicles do not settle unless they are subjected to centrifugation.

While conditions in a pharmaceutical suspension are not in strict accord with those laid down for Stokes' law, Eq 35, provides those factors that can be expected to influence the rate of settling. Thus, sedimentation velocity will be reduced by decreasing the particle size, provided the particles are kept in a deflocculated state. The rate of sedimentation will be an inverse function of the viscosity of the dispersion medium. However, too high a viscosity is undesirable, especially if the suspending medium is Newtonian rather than shear-thinning (see Chapter 20), since it then becomes difficult to redisperse material which has settled. It also may be inconvenient to remove a viscous suspension from its container. When the size of particles undergoing sedimentation is reduced to approximately 2 μm, random Brownian movement is observed and the rate of sedimentation departs markedly from the theoretical predictions of Stokes' law. The actual size at which Brownian movement becomes significant depends on the density of the particle as well as the viscosity of the dispersion medium.

Flocculation and Deflocculation—Zeta potential ψ_z is a measurable indication of the potential existing at the surface of a particle. When ψ_z is relatively high (25 mV or more), the repulsive forces between two particles exceed the attractive London forces. Accordingly, the particles are dispersed and are said to be *deflocculated*. Even when brought close together by random motion or agitation, deflocculated particles resist collision due to their high surface potential.

The addition of a preferentially adsorbed ion whose charge is opposite in sign to that on the particle leads to a progressive lowering of ψ_z. At some concentration of the added ion the electrical forces of repulsion are lowered sufficiently that the forces of attraction predominate. Under these conditions the particles may approach each other more closely and form loose aggregates, termed flocs. Such a system is said to be *flocculated*.

Some workers restrict the term *flocculation* to the aggregation brought about by chemical bridging; aggregation involving a reduction of repulsive potential at the double layer is referred to as *coagulation*. Other workers regard flocculation as aggregation in the secondary minimum of the potential energy curve of two interacting particles and coagulation as aggregation in the primary minimum. In the present chapter the term *flocculation* is used for all aggregation processes, irrespective of mechanism.

The continued addition of the flocculating agent can reverse the above process, if the zeta potential increases sufficiently in the opposite direction. Thus, the adsorption of anions onto positively charged deflocculated particles in suspension will lead to flocculation. The addition of more anions can eventually generate a net negative charge on the particles. When this has achieved the required magnitude, deflocculation may occur again. The only difference from the starting system is that the net charge on the particles in their deflocculated state is negative rather than positive.

Table XV—Relative Properties of Flocculated and Deflocculated Particles in Suspension

	Deflocculated	Flocculated
1.	Particles exist in suspension as separate entities.	Particles form loose aggregates.
2.	Rate of sedimentation is slow, since each particle settles separately and particle size is minimal.	Rate of sedimentation is high, since particles settle as a floc, which is a collection of particles.
3.	A sediment is formed slowly.	A sediment is formed rapidly.
4.	The sediment eventually becomes very closely packed, due to weight of upper layers of sedimenting material. Repulsive forces between particles are overcome and a hard cake is formed which is difficult, if not impossible, to redisperse.	The sediment is loosely packed and possesses a scaffold-like structure. Particles do not bond tightly to each other and a hard, dense cake does not form. The sediment is easy to redisperse, so as to reform the original suspension.
5.	The suspension has a pleasing appearance, since the suspended material remains suspended for a relatively long time. The supernatant also remains cloudy, even when settling is apparent.	The suspension is somewhat unsightly, due to rapid sedimentation and the presence of an obvious, clear supernatant region. This can be minimized if the volume of sediment is made large. Ideally, volume of sediment should encompass the volume of the suspension.

Some of the major differences between suspensions of flocculated and deflocculated particles are presented in Table XV.

Effect of Flocculation—In a deflocculated system containing a distribution of particle sizes, the larger particles naturally settle faster than the smaller particles. The very small particles remain suspended for a considerable length of time, with the result that no distinct boundary is formed between the supernatant and the sediment. Even when a sediment becomes discernible, the supernatant remains cloudy.

When the same system is flocculated (in a manner to be discussed later), two effects are immediately apparent. First, the flocs tend to fall together so that a distinct boundary between the sediment and the supernatant is readily observed; second, the supernatant is clear, showing that the very fine particles have been incorporated into the flocs. The initial rate of settling in flocculated systems is determined by the size of the flocs and the porosity of the aggregated mass. Under these circumstances it is perhaps better to use the term *subsidence*, rather than sedimentation.

Quantitative Expressions of Sedimentation and Flocculation

Frequently, the pharmacist needs to assess a formulation in terms of the amount of flocculation in the suspension and to compare this with that found in other formulations. The two parameters commonly used for this purpose are outlined below.

Sedimentation Volume—The *sedimentation volume*, F, is the ratio of the equilibrium volume of the sediment, V_u, to the total volume of the suspension, V_0. Thus,

$$F = V_u/V_0 \quad (36)$$

As the volume of suspension which appears occupied by the sediment increases, the value of F, which normally ranges from nearly 0 to 1, increases. In the system where $F = 0.75$, for example, 75% of the total volume in the container is apparently occupied by the loose, porous flocs forming the sediment. This is illustrated in Fig 19-33. When $F = 1$, no sediment is apparent even though the system is flocculated. This is the ideal suspension for, under these conditions, no sedimentation will occur. Caking also will be absent. Furthermore, the suspension is esthetically pleasing, there being no visible, clear supernatant.

Degree of Flocculation—A better parameter for comparing flocculated systems is the *degree of flocculation*, β, which relates the sedimentation volume of the flocculated suspension, F, to the sedimentation volume of the suspension when deflocculated, F_∞. It is expressed as

$$\beta = F/F_\infty \quad (37)$$

The degree of flocculation is, therefore, an expression of the increased sediment volume resulting from flocculation.

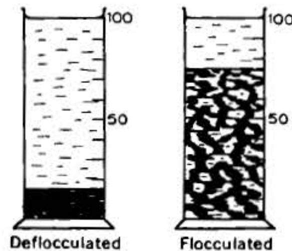


Fig 19-33. Sedimentation parameters of suspensions. Deflocculated suspension: $F_\infty = 0.15$ Flocculated suspension: $F = 0.75$; $\beta = 5.0$.

If, for example, β has a value of 5.0 (Fig 19-33), this means that the volume of sediment in the flocculated system is five times that in the deflocculated state. If a second flocculated formulation results in a value for β of say 6.5, this latter suspension obviously is preferred, if the aim is to produce as flocculated a product as possible. As the degree of flocculation in the system decreases, β approaches unity, the theoretical minimum value.

Suspensions and their Formulation

A pharmaceutical suspension may be defined as a coarse dispersion containing finely divided insoluble material suspended in a liquid medium. Suspension dosage forms are given by the oral route, injected intramuscularly or subcutaneously, applied to the skin in topical preparations, and used ophthalmically in the eye. They are an important class of dosage form. Since some products are occasionally prepared in a dry form, to be placed in suspension at the time of dispensing by the addition of an appropriate vehicle, this definition is extended to include these products.

There are certain criteria that a well-formulated suspension should meet. The dispersed particles should be of such a size that they do not settle rapidly in the container. However, in the event that sedimentation occurs, the sediment must not form a hard cake. Rather, it must be capable of redispersion with a minimum effort on the part of the patient. Additionally, the product should be easy to pour, pleasant to take, and resistant to microbial attack.

The three major problem areas associated with suspensions are (1) adequate dispersion of the particles in the vehicle, (2) settling of the dispersed particles, and (3) caking of these particles in the sediment so as to resist redispersion. Much of the following discussion will deal with the factors that influence these processes and the ways in which they can be minimized.

The formulation of a suspension possessing optimal physical stability depends on whether the particles in suspension are to be flocculated or to remain deflocculated. One approach involves use of a structured vehicle to keep deflocculated particles in suspension; a second depends on controlled flocculation as a means of preventing cake formation. A

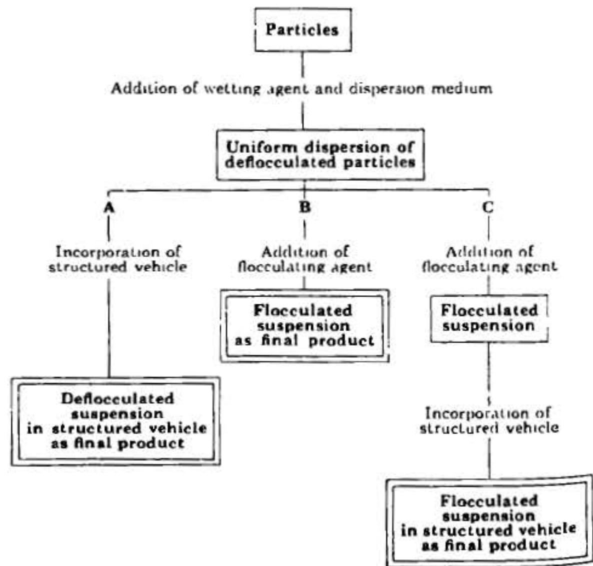


Fig 19-34. Alternative approaches to the formulation of suspensions.

third, a combination of the two previous methods, results in a product with optimum stability. The various schemes are illustrated in Fig 19-34.

Dispersion of Particles—The dispersion step has been discussed earlier in this chapter. Surface-active agents commonly are used as wetting agents; maximum efficiency is obtained when the HLB value lies within the range of 7 to 9. A concentrated solution of the wetting agent in the vehicle may be used to prepare a slurry of the powder; this is diluted with the required amount of vehicle. Alcohol and glycerin may be used sometimes in the initial stages to disperse the particles, thereby allowing the vehicle to penetrate the powder mass.

Only the minimum amount of wetting agent should be used, compatible with producing an adequate dispersion of the particles. Excessive amounts may lead to foaming or impart an undesirable taste or odor to the product. Invariably, as a result of wetting, the dispersed particles in the vehicle are deflocculated.

Structured Vehicles—Structured vehicles are generally aqueous solutions of polymeric materials, such as the hydrocolloids, which are usually negatively charged in aqueous solution. Typical examples are methylcellulose, carboxymethylcellulose, bentonite, and Carbopol. The concentration employed will depend on the consistency desired for the suspension which, in turn, will relate to the size and density of the suspended particles. They function as viscosity-imparting suspending agents and, as such, reduce the rate of sedimentation of dispersed particles.

The rheological properties of suspending agents are considered elsewhere (Chapter 20). Ideally, these form pseudoplastic or plastic systems which undergo shear-thinning. Some degree of thixotropy is also desirable. Non-Newtonian materials of this type are preferred over Newtonian systems because, if the particles eventually settle to the bottom of the container, their redispersion is facilitated by the vehicle thinning when shaken. When the shaking is discontinued, the vehicle regains its original consistency and the redispersed particles are held suspended. This process of redispersion, facilitated by a shear-thinning vehicle, presupposes that the deflocculated particles have not yet formed a cake. If sedimentation and packing have proceeded to the point where considerable caking has occurred, redispersion is virtually impossible.

Controlled Flocculation—When using this approach (see Fig 19-34, B and C), the formulator takes the deflocculated, wetted dispersion of particles and attempts to bring about flocculation by the addition of a flocculating agent; most commonly, these are either electrolytes, polymers, or surfactants. The aim is to *control* flocculation by adding that amount of flocculating agent which results in the maximum sedimentation volume.

Electrolytes are probably the most widely used flocculating agents. They act by reducing the electrical forces of repulsion between particles, thereby allowing the particles to form the loose flocs so characteristic of a flocculated suspension. Since the ability of particles to come together and form a floc depends on their surface charge, zeta potential measurements on the suspension, as an electrolyte is added, provide valuable information as to the extent of flocculation in the system.

This principle is illustrated by reference to the following example, taken from the work of Haines and Martin.[50] Particles of sulfamerazine in water bear a negative charge. The serial addition of a suitable electrolyte, such as aluminum chloride, causes a progressive reduction in the zeta potential of the particles. This is due to the preferential adsorption of the trivalent aluminum cation. Eventually, the zeta potential will reach zero and then become positive as the addition of $AlCl_3$ is continued.

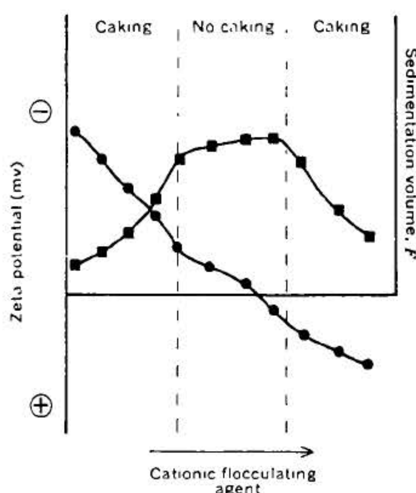


Fig 19-35. Typical relationship between caking, zeta potential and sedimentation volume, as a positively charged flocculating agent is added to a suspension of negatively charged particles. ●: zeta potential; ■: sedimentation volume

If sedimentation studies are run simultaneously on suspensions containing the same range of $AlCl_3$ concentrations, a relationship is observed (Fig 19-35) between the sedimentation volume, F, the presence or absence of caking, and the zeta potential of the particles. In order to obtain a flocculated, noncaking suspension with the maximum sedimentation volume, the zeta potential must be controlled so as to lie within a certain range (generally less than 25 mV). This is achieved by the judicious use of an electrolyte.

A comparable situation is observed when a negative ion such as PO_4^{3-} is added to a suspension of positively charged particles such as bismuth subnitrate. Ionic and nonionic surfactants and lyophilic polymers also have been used to flocculate particles in suspension. Polymers, which act by forming a "bridge" between particles, may be the most efficient additives for inducing flocculation. Thus, it has been shown that the sedimentation volume is higher in suspensions flocculated with an anionic heteropolysaccharide than when electrolytes were used.

Work by Matthews and Rhodes,[51-53] involving both experimental and theoretical studies, has confirmed the formulation principles proposed by Martin and Haines. The suspensions used by Matthews and Rhodes contained 2.5% w/v of griseofulvin as a fine powder together with the anionic surfactant sodium dioxyethylated dodecyl sulfate (10^{-3} molar) as a wetting agent. Increasing concentrations of aluminum chloride were added and the sedimentation height (equivalent to the sedimentation volume, see page 295) and the zeta potential recorded. Flocculation occurred when a concentration of 10^{-3} molar aluminum chloride was reached. At this point the zeta potential had fallen from −46.4 mV to −17.0 mV. Further reduction of the zeta potential, to −4.5 mV by use of 10^{-2} molar aluminum chloride did not increase sedimentation height, in agreement with the principles shown in Fig 19-35.

Matthews and Rhodes then went on to show, by computer analysis, that the DLVO theory (see page 285) predicted the results obtained, namely, that the griseofulvin suspensions under investigation would remain deflocculated when the concentration of aluminum chloride was 10^{-4} molar or less. Only at concentrations in the range of 10^{-3} to 10^{-2} molar aluminum chloride did the theoretical plots show deep primary minima, indicative of flocculation. These occurred at a distance of separation between particles of approximately

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50 Å, and led Matthews and Rhodes to conclude that coagulation had taken place in the primary minimum.

Schneider, *et al*[54] have published details of a laboratory investigation (suitable for undergraduates) that combines calculations based on the DLVO theory carried out with an interactive computer program with actual sedimentation experiments performed on simple systems.

Flocculation in Structured Vehicles—The ideal formulation for a suspension would seem to be when flocculated particles are supported in a structured vehicle.

As shown in Fig 19-34 (under C), the process involves dispersion of the particles and their subsequent flocculation. Finally, a lyophilic polymer is added to form the structured vehicle. In developing the formulation, care must be taken to ensure the absence of any incompatibility between the flocculating agent and the polymer used for the structured vehicle. A limitation is that virtually all the structured vehicles in common use are hydrophilic colloids and carry a negative charge. This means that an incompatibility arises if the charge on the particles is originally negative. Flocculation in this instance requires the addition of a positively charged flocculating agent or ion; in the presence of such a material, the negatively charged suspending agent may coagulate and lose its suspendability. This situation does not arise with particles that bear a positive charge, as the negative flocculating agent which the formulator must employ is compatible with the similarly charged suspending agent.

Chemical Stability of Suspensions—Particles that are completely insoluble in a liquid vehicle are unlikely to undergo most chemical reactions leading to degradation. However, most drugs in suspension have a finite solubility, even though this may be of the order of fractions of a microgram per mL. As a result, the material in solution may be susceptible to degradation. However, Tingstad and coworkers[55] developed a simplified method for determining the stability of drugs in suspension. The approach is based on the assumptions that (1) degradation takes place only in the solution and is first order, (2) the effect of temperature on drug solubility and reaction rate conforms with classical theory, and (3) dissolution is not rate-limiting on degradation.

Preparation of Suspensions—The small-scale preparation of suspensions may be readily undertaken by the practicing pharmacist with the minimum of equipment. The initial dispersion of the particles is best carried out by trituration in a mortar, the wetting agent being added in small increments to the powder. Once the particles have been wetted adequately, the slurry may be transferred to the final container. The next step depends on whether the deflocculated particles are to be suspended in a structured vehicle, flocculated, or flocculated and then suspended. Regardless of which of the alternative procedures outlined in Fig 19-34 is employed, the various manipulations can be carried out easily in the bottle, especially if an aqueous solution of the suspending agent has been prepared beforehand.

For a detailed discussion of the methods used in the large-scale production of suspensions, see the relevant section in Chapter 82.

Emulsions in Pharmacy

An emulsion is a dispersed system containing at least two immiscible liquid phases. The majority of conventional emulsions in pharmaceutical use have dispersed particles ranging in diameter from 0.1 to 100 μm. As with suspensions, emulsions are thermodynamically unstable as a result of the excess free energy associated with the surface of the droplets. The dispersed droplets, therefore, strive to come together and reduce the surface area. In addition to this flocculation effect, also observed with suspensions, the dispersed particles can coalesce, or fuse, and this can result in the eventual destruction of the emulsion. In order to minimize this effect a third component, the *emulsifying agent*, is added to the system to improve its stability. The choice of emulsifying agent is critical to the preparation of an emulsion possessing optimum stability. The efficiency of present-day emulsifiers permits the preparation of emulsions which are stable for many months and even years, even though they are thermodynamically unstable.

Emulsions are widely used in pharmacy and medicine, and emulsified materials can possess advantages not observed when formulated in other dosage forms. Thus, certain medicinal agents having an objectionable taste have been made more palatable for oral administration when formulated in an emulsion. The principles of emulsification have been applied extensively in the formulation of dermatological creams and lotions. Intravenous emulsions of contrast media have been developed to assist the physician in undertaking X-ray examinations of the body organs while exposing the patient to the minimum of radiation. Considerable attention has been directed towards the use of sterile, stable intravenous emulsions containing fat, carbohydrate, and vitamins all in one preparation. Such products are administered to patients unable to assimilate these vital materials by the normal oral route.

Emulsions offer potential in the design of systems capable of giving controlled rates of drug release and of affording protection to drugs susceptible to oxidation or hydrolysis. There is still a need for well-characterized dermatological products with reproducible properties, regardless of whether these products are antibacterial, sustained-release, protective, or emollient lotions, creams or ointments. The principle of emulsification is involved in an increasing number of aerosol products.

The pharmacist must be familiar with the types of emulsions and the properties and theories underlying their preparation and stability; such is the purpose of the remainder of this chapter. Microemulsions, which can be regarded as isotropic, swollen micellar systems are discussed in Chapter 83.

Emulsion Type and Means of Detection

A stable emulsion must contain at least three components; namely, the dispersed phase, the dispersion medium, and the emulsifying agent. Invariably, one of the two immiscible liquids is aqueous while the second is an oil. Whether the aqueous or the oil phase becomes the dispersed phase depends primarily on the emulsifying agent used and the relative amounts of the two liquid phases. Hence, an emulsion in which the oil is dispersed as droplets throughout the aqueous phase is termed an oil-in-water, O/W, emulsion. When water is the dispersed phase and an oil the dispersion medium, the emulsion is of the water-in-oil, W/O, type. Most pharmaceutical emulsions designed for oral administration are of the O/W type; emulsified lotions and creams are either O/W or W/O, depending on their use. Butter and salad creams are W/O emulsions.

Recently, so-called *multiple* emulsions have been developed with a view to delaying the release of an active ingredient. In these types of emulsions three phases are present, ie, the emulsion has the form W/O/W or O/W/O. In these

"emulsions within emulsions," any drug present in the innermost phase must now cross two phase boundaries to reach the external, continuous, phase.

It is important for the pharmacist to know the type of emulsion he has prepared or is dealing with, since this can affect its properties and performance. Unfortunately, the several methods available can give incorrect results, and so the type of emulsion determined by one method should always be confirmed by means of a second method.

Dilution Test—This method depends on the fact that an O/W emulsion can be diluted with water and a W/O emulsion with oil. When oil is added to an O/W emulsion or water to a W/O emulsion, the additive is not incorporated into the emulsion and separation is apparent. The test is greatly improved if the addition of the water or oil is observed microscopically.

Conductivity Test—An emulsion in which the continuous phase is aqueous can be expected to possess a much higher conductivity than an emulsion in which the continuous phase is an oil. Accordingly, it frequently happens that when a pair of electrodes, connected to a lamp and an electrical source, are dipped into an O/W emulsion, the lamp lights due to passage of a current between the two electrodes. If the lamp does not light, it is assumed that the system is W/O.

Dye-Solubility Test—The knowledge that a water-soluble dye will dissolve in the aqueous phase of an emulsion while an oil-soluble dye will be taken up by the oil phase provides a third means of determining emulsion type. Thus, if microscopic examination shows that a water-soluble dye has been taken up by the continuous phase, we are dealing with an O/W emulsion. If the dye has not stained the continuous phase, the test is repeated using a small amount of an oil-soluble dye. Coloring of the continuous phase confirms that the emulsion is of the W/O type.

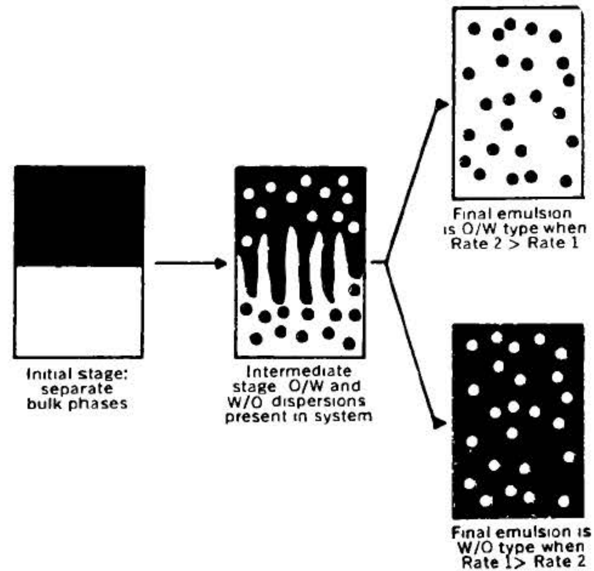


Fig 19-36. Effect of rate of coalescence on emulsion type. Rate 1: O/W coalescence rate; Rate 2: W/O coalescence rate. ●: oil; O: water. For an explanation of Rates 1 and 2, refer to the discussion of Davies on page 304.

Formation and Breakdown of Dispersed Liquid Droplets

An emulsion exists as the result of two competing processes, namely, the dispersion of one liquid throughout another as droplets, and the combination of these droplets to reform the initial bulk liquids. The first process increases the free energy of the system, while the second works to reduce the free energy. Accordingly, the second process is spontaneous and continues until breakdown is complete; ie, the bulk phases are reformed.

It is of little use to form a well-dispersed emulsion if it quickly breaks down. Similarly, unless adequate attention is given to achieving an optimum dispersion during preparation, the stability of an emulsion system may be compromised from the start. Dispersion is brought about by well-designed and well-operated machinery, capable of producing droplets in a relatively short period of time. Such equipment is discussed in Chapter 83. The reversal back to the bulk phases is minimized by utilizing those parameters which influence the stability of the emulsion once it is formed.

Dispersion Process To Form Droplets—Consider two immiscible liquid phases in a test tube. In order to disperse one liquid as droplets within the other, the interface between the two liquids must be disturbed and expanded to a sufficient degree so that "fingers" or threads of one liquid pass into the second liquid, and *vice versa*. These threads are unstable, and become varicosed or beaded. The beads separate and become spherical, as illustrated in Fig 19-36. Depending on the agitation or the shear rate used, larger droplets are also deformed to give small threads, which in turn produce smaller drops.

The time of agitation is important. Thus, the mean size of droplets decreases rapidly in the first few seconds of agitation. The limiting size range is generally reached within 1 to 5 minutes, and results from the number of droplets coalescing being equivalent to the number of new droplets being formed. It is uneconomical to continue agitation any further.

The liquids may be agitated or sheared by several means. Shaking is commonly employed, especially when the components are of low viscosity. Intermittent shaking is frequently more efficient than continual shaking, possibly because the short time interval between shakes allows the thread which is forced across the interface time to break down into drops which are then isolated in the opposite phase. Continuous, rapid agitation tends to hinder this breakdown to form drops. A mortar and pestle is employed frequently in the extemporaneous preparation of emulsions. It is not a very efficient technique and is not used on a large scale. Improved dispersions are achieved by the use of high-speed mixers, blenders, colloid mills and homogenizers. Ultrasonic techniques also have been employed and are described in Chapter 83.

The phenomenon of spontaneous emulsification, as the name implies, occurs without any external agitation. There is, however, an internal agitation arising from certain physicochemical processes that affect the interface between the two bulk liquids. For a description of this process, see Davies and Rideal in the *Bibliography*.

Coalescence of Droplets—Coalescence is a process distinct from flocculation (aggregation), which commonly precedes it. While flocculation is the clumping together of particles, coalescence is the fusing of the agglomerates into a larger drop, or drops. Coalescence is usually rapid when two immiscible liquids are shaken together, since there is no large energy barrier to prevent fusion of drops and reformation of the original bulk phases. When an emulsifying agent is added to the system, flocculation still may occur but coalescence is reduced to an extent depending on the efficacy of the emulsifying agent to form a stable, coherent interfacial film. It is therefore possible to prepare emulsions that are flocculated, yet which do not coalesce. In addition to the interfacial film around the droplets acting as a mechanical

barrier, the drops also are prevented from coalescing by the presence of a thin layer of continuous phase between particles clumped together.

Davies[56] showed the importance of coalescence rates in determining emulsion type; this work is discussed in more detail on page 304.

Emulsifying Agent

The process of coalescence can be reduced to insignificant levels by the addition of a third component—the emulsifying agent or emulsifier. The choice of emulsifying agent is frequently critical in developing a successful emulsion, and the pharmacist should be aware of

The desirable properties of emulsifying agents.
How different emulsifiers act to optimize emulsion stability.
How the type and physical properties of the emulsion can be affected by the emulsifying agent.

Desirable Properties

Some of the desirable properties of an emulsifying agent are that it should

1. Be surface-active and reduce surface tension to below 10 dynes/cm.
2. Be adsorbed quickly around the dispersed drops as a condensed, nonadherent film which will prevent coalescence.
3. Impart to the droplets an adequate electrical potential so that mutual repulsion occurs.
4. Increase the viscosity of the emulsion.
5. Be effective in a reasonably low concentration.

Not all emulsifying agents possess these properties to the same degree; in fact, not every good emulsifier necessarily possesses all these properties. Further, there is no one "ideal" emulsifying agent because the desirable properties of an emulsifier depend, in part, on the properties of the two immiscible phases in the particular system under consideration.

Interfacial Tension—Lowering of interfacial tension is one way in which the increased surface free energy associated with the formation of droplets, and hence surface area, in an emulsion can be reduced (Eq 29). Assuming the droplets to be spherical, it can be shown that

$$\Delta F = \frac{6\gamma V}{d} \qquad (38)$$

where V is the volume of dispersed phase in mL and d is the mean diameter of the particles. In order to disperse 100 mL of oil as 1-μm (10^{-4}-cm) droplets in water when $\gamma_{O/W} = 50$ dynes/cm, requires an energy input of

$$\Delta F = \frac{6 \times 50 \times 100}{1 \times 10^{-4}} = 30 \times 10^{7} \text{ ergs}$$

$$= 30 \text{ joules or } 30/4.184 = 7.2 \text{ cal}$$

In the above example the addition of an emulsifier that will reduce γ from 50 to 5 dynes/cm will reduce the surface free energy from 7.2 to around 0.7 cal. Likewise, if the interfacial tension is reduced to 0.5 dyne/cm, a common occurrence, the original surface free energy is reduced a hundredfold. Such a reduction can help to maintain the surface area generated during the dispersion process.

Film Formation—The major requirement of a potential emulsifying agent is that it readily form a film around each droplet of dispersed material. The main purpose of this film—which can be a monolayer, a multilayer, or a collection of small particles adsorbed at the interface—is to form a barrier which prevents the coalescence of droplets that come into contact with one another. For the film to be an efficient barrier, it should possess some degree of surface elasticity and should not thin out and rupture when sandwiched between two droplets. If broken, the film should have the capacity to reform rapidly.

Electrical Potential—The origin of an electrical potential at the surface of a droplet has been discussed earlier in the chapter. Insofar as emulsions are concerned, the presence of a well-developed charge on the droplet surface is significant in promoting stability by causing repulsion between approaching drops. This potential is likely to be greater when an ionized emulsifying agent is employed.

Concentration of Emulsifier—The main objective of an emulsifying agent is to form a condensed film around the droplets of the dispersed phase. An inadequate concentration will do little to prevent coalescence. Increasing the emulsifier concentration above an optimum level achieves little in terms of increased stability. In practice the aim is to use the minimum amount consistent with producing a satisfactory emulsion.

It frequently helps to have some idea of the amount of emulsifier required to form a condensed film, one molecule thick, around each droplet. Suppose we wish to emulsify 50 g of an oil, density = 1.0, in 50 g of water. The desired particle diameter is 1 μm. Thus,

Particle diameter = 1 μm = 1×10^{-4} cm

Volume of particle $= \frac{\pi d^3}{6} = 0.524 \times 10^{-12}$ cm^3

Total number of particles in 50 g

$$= \frac{50}{0.524 \times 10^{-12}} = 95.5 \times 10^{12}$$

Surface area of each particle $= \pi d^2 = 3.142 \times 10^{-8}$ cm^2

Total surface area $= 3.142 \times 10^{-8}$

$$\times 95.5 \times 10^{12} = 300 \times 10^{4} \text{ cm}^2$$

If the area each molecule occupies at the oil/water interface is 30 Å^2 (30×10^{-16} cm^2), we require

$$\frac{300 \times 10^4}{30 \times 10^{16}} = 1 \times 10^{21} \text{ molecules}$$

A typical emulsifying agent might have a molecular weight of 1000. Thus, the required weight is

$$\frac{1000 \times 10^{21}}{6.023 \times 10^{23}} = 1.66 \text{ g}$$

To emulsify 10 g of oil would require 0.33 g of the emulsifying agent, etc. While the approach is an oversimplification of the problem, it does at least allow the formulator to make a reasonable estimate of the required concentration of emulsifier.

Emulsion Rheology—The emulsifying agent and other components of an emulsion can affect the rheologic behavior of an emulsion in several ways and these are summarized in Table XVI. It should be borne in mind that the droplets of the internal phase are deformable under shear and that the adsorbed layer of emulsifier affects the interactions between adjacent droplets and also between a droplet and the continuous phase.

The means by which the rheological behavior of emulsions can be controlled have been discussed by Rogers.[58]

Mechanism of Action

Emulsifying agents may be classified in accordance with the type of film they form at the interface between the two phases.

Monomolecular Films—Those surface-active agents which are capable of stabilizing an emulsion do so by form-

Table XVI—Factors Influencing Emulsion Viscosity[57]

1. Internal phase
   a. Volume concentration (ϕ); hydrodynamic interaction between globules; flocculation, leading to formation of globule aggregates.
   b. Viscosity (η_i); deformation of globules in shear.
   c. Globule size, and size distribution, technique used to prepare emulsion; interfacial tension between the two liquid phases: globule behavior in shear; interaction with continuous phase; globule interaction.
   d. Chemical constitution.
2. Continuous phase
   a. Viscosity (η_0), and other rheological properties.
   b. Chemical constitution, polarity, pH; potential energy of interaction between globules.
   c. Electrolyte concentration if polar medium.
3. Emulsifying agent
   a. Chemical constitution; potential energy of interaction between globules.
   b. Concentration, and solubility in internal and continuous phases; emulsion type; emulsion inversion; solubilization of liquid phases in micelles.
   c. Thickness of film adsorbed around globules, and its rheological properties, deformation of globules in shear; fluid circulation within globules.
   d. Electroviscous effect.
4. Additional stabilizing agents
   Pigments, hydrocolloids, hydrous oxides; effect on rheologic properties of liquid phases, and interfacial boundary region.

ing a monolayer of adsorbed molecules or ions at the oil/water interface (Fig 19-37). In accordance with Gibbs' law (Eq 29) the presence of an interfacial excess necessitates a reduction in interfacial tension. This results in a more stable emulsion because of a proportional reduction in the surface free energy. Of itself, this reduction is probably not the main factor promoting stability. More significant is the fact that the droplets are surrounded now by a coherent monolayer which prevents coalescence between approaching droplets. If the emulsifier forming the monolayer is ionized, the presence of strongly charged and mutually repelling droplets increases the stability of the system. With unionized, nonionic surface-active agents, the particles may still carry a charge; this arises from adsorption of a specific ion or ions from solution.

Multimolecular Films—Hydrated lyophilic colloids form multimolecular films around droplets of dispersed oil (Fig 19-37). The use of these agents has declined in recent years because of the large number of synthetic surface-active agents available which possess well-marked emulsifying properties. While these hydrophilic colloids are adsorbed at an interface (and can be regarded therefore as "surface-active"), they do not cause an appreciable lowering in surface tension. Rather, their efficiency depends on their ability to form strong, coherent multimolecular films. These act as a coating around the droplets and render them highly resistant to coalescence, even in the absence of a well-developed surface potential. Furthermore, any hydrocolloid not adsorbed at the interface increases the viscosity of the continuous aqueous phase; this enhances emulsion stability.

Solid Particle Films—Small solid particles that are wetted to some degree by both aqueous and nonaqueous liquid phases act as emulsifying agents. If the particles are too hydrophilic, they remain in the aqueous phase; if too hydrophobic, they are dispersed completely in the oil phase. A second requirement is that the particles are small in relation to the droplets of the dispersed phase (Fig 19-37).

Chemical Types

Emulsifying agents may also be classified in terms of their chemical structure; there is some correlation between this

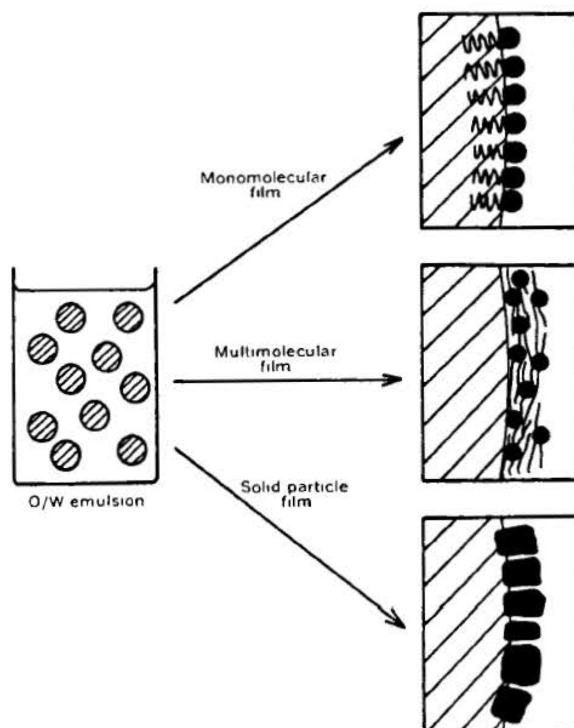


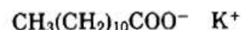
Fig 19-37. Types of films formed by emulsifying agents at the oil/water interface. Orientations are shown for O/W emulsions. ▨: oil; □: water.

classification and that based on the mechanism of action. For example, the majority of emulsifiers forming monomolecular films are synthetic, organic materials. Most of the emulsifiers that form multimolecular films are obtained from natural sources and are organic. A third group is composed of solid particles, invariably inorganic, that form films composed of finely divided solid particles.

Accordingly, the classification adopted divides emulsifying agents into *synthetic*, *natural*, and *finely dispersed solids* (Table XVII). A fourth group, the *auxiliary materials* (Table XVIII), are weak emulsifiers. The agents listed are designed to illustrate the various types available; they are not meant to be exhaustive.

Synthetic Emulsifying Agents—This group of surface-active agents which act as emulsifiers may be subdivided into anionic, cationic, and nonionic, depending on the charge possessed by the surfactant.

Anionics—In this subgroup the surfactant ion bears a negative charge. The potassium, sodium, and ammonium salts of lauric and oleic acid are soluble in water and are good O/W emulsifying agents. They do, however, have a disagreeable taste and are irritating to the gastrointestinal tract; this limits them to emulsions prepared for external use. Potassium laurate, a typical example, has the structure



$$CH_3(CH_2)_{10}COO^- \quad K^+$$

Solutions of alkali soaps have a high pH; they start to precipitate out of solution below pH 10 because the unionized fatty acid is now formed, and this has a low aqueous solubility. Further, the free fatty acid is ineffective as an emulsifier and so emulsions formed from alkali soaps are not stable at pH values less than about 10.

The calcium, magnesium and aluminum salts of fatty acids, often termed the metallic soaps, are water insoluble and result in W/O emulsions.

Table XVII—Classification of Emulsifying Agents

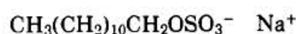
Type	Type of film	Examples
Synthetic (surface-active agents)	Monomolecular	*Anionic*
		Soaps
		Potassium laurate
		Triethanolamine stearate
		Sulfates
		Sodium lauryl sulfate
		Alkyl polyoxyethylene sulfates
		Sulfonates
		Dioctyl sodium sulfosuccinate
		Cationic:
		Quaternary ammonium compounds
		Cetyltrimethylammonium bromide
		Lauryldimethylbenzylammonium chloride
		Nonionic.
		Polyoxyethylene fatty alcohol ethers
		Sorbitan fatty acid esters
		Polyoxyethylene sorbitan fatty acid esters
Natural	Multimolecular	*Hydrophilic colloids.*
		Acacia
		Gelatin
	Monomolecular	Lecithin
		Cholesterol
Finely divided solids	Solid particle	*Colloidal clays*
		Bentonite
		Veegum
		Metallic hydroxides:
		Magnesium hydroxide

Table XVIII—Auxiliary Emulsifying Agents[55]

Product	Source and composition	Principal use
Bentonite	Colloidal hydrated aluminum silicate	Hydrophilic thickening agent and stabilizer for O/W and W/O lotions and creams
Cetyl alcohol	Chiefly $C_{16}H_{33}OH$	Lipophilic thickening agent and stabilizer for O/W lotions and ointments
Glyceryl monostearate	$C_{17}H_{35}COOCH_2CHOHCH_2OH$	Lipophilic thickening agent and stabilizer for O/W lotions and ointments
Methylcellulose	Series of methyl esters of cellulose	Hydrophilic thickening agent and stabilizer for O/W emulsions; weak O/W emulsifier
Sodium alginate	The sodium salt of alginic acid, a purified carbohydrate extracted from giant kelp	Hydrophilic thickening agent and stabilizer for O/W emulsions
Sodium carboxymethylcellulose	Sodium salt of the carboxymethyl esters of cellulose	Hydrophilic thickening agent and stabilizer for O/W emulsions
Stearic acid	A mixture of solid acids from fats, chiefly stearic and palmitic	Lipophilic thickening agent and stabilizer for O/W lotions and ointments. Forms a true emulsifier when reacted with an alkali
Stearyl alcohol	Chiefly $C_{18}H_{37}OH$	Lipophilic thickening agent and stabilizer for O/W lotions and ointments
Veegum	Colloidal magnesium aluminum silicate	Hydrophilic thickening agent and stabilizer for O/W lotions and creams

Another class of soaps are salts formed from a fatty acid and an organic amine such as triethanolamine. While these O/W emulsifiers are also limited to external preparations, their alkalinity is considerably less than that of the alkali soaps and they are active as emulsifiers down to around pH 8. These agents are less irritating than the alkali soaps.

Sulfated alcohols are neutralized sulfuric acid esters of such fatty alcohols as lauryl and cetyl alcohol. These compounds are an important group of pharmaceutical surfactants. They are used chiefly as wetting agents, although they do have some value as emulsifiers, particularly, when used in conjunction with an auxiliary agent. A frequently used compound is sodium lauryl sulfate.



$$CH_3(CH_2)_{10}CH_2OSO_3^- \quad Na^+$$

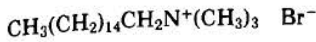
Sulfonates are a class of compounds in which the sulfur atom is connected directly to the carbon atom, giving the general formula

$$CH_3(CH_2)_nCH_2SO_3^- \quad Na^+$$

Sulfonates have a higher tolerance to calcium ions and do not hydrolyze as readily as the sulfates. A widely used surfactant of this type is dioctyl sodium sulfosuccinate.

Cationics—The surface activity in this group resides in the positively charged cation. These compounds have marked bactericidal properties. This makes them desirable in emulsified anti-infective products such as skin lotions and creams. The pH of an emulsion prepared with a cationic emulsifier lies in the pH 4–6 range. Since this includes the normal pH of the skin, cationic emulsifiers are advantageous in this regard also.

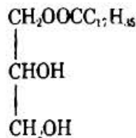
Cationic agents are weak emulsifiers and are generally formulated with a stabilizing or auxiliary emulsifying agent such as cetostearyl alcohol. The only group of cationic agents used extensively as emulsifying agents are the quaternary ammonium compounds. An example is cetyltrimethylammonium bromide.



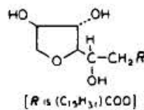
Cationic emulsifiers should not be used in the same formulation with anionic emulsifiers as they will interact. While the incompatibility may not be immediately apparent as a precipitate, virtually all of the desired antibacterial activity will generally have been lost.

Nonionics—These undissociated surfactants find widespread use as emulsifying agents when they possess the proper balance of hydrophilic and lipophilic groups within the molecule. Their popularity is based on the fact that, unlike the anionic and cationic types, nonionic emulsifiers are not susceptible to pH changes and the presence of electrolytes. The number of nonionic agents available is legion; the most frequently used are the glyceryl esters, polyoxyethylene glycol esters and ethers, and the sorbitan fatty acid esters and their polyoxyethylene derivatives.

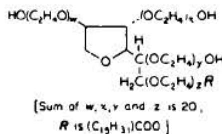
A glyceryl ester, such as glyceryl monostearate, is too lipophilic to serve as a good emulsifier; it is widely used as an auxiliary agent (Table XVIII) and has the structure



Sorbitan fatty acid esters, such as sorbitan monopalmitate



are nonionic oil-soluble emulsifiers that promote W/O emulsions. The polyoxyethylene sorbitan fatty acid esters, such as polyoxyethylene sorbitan monopalmitate, are hydrophilic water-soluble derivatives that favor O/W emulsions.



Polyoxyethylene glycol esters, such as the monostearate, $C_{17}H_{35}COO(CH_2OCH_2)_nH$, also are used widely.

Very frequently, the best results are obtained from blends of nonionic emulsifiers. Thus, an O/W emulsifier customarily will be used in an emulsion with a W/O emulsifier. When blended properly, the nonionics produce fine-textured stable emulsions.

Natural Emulsifying Agents—Of the numerous emulsifying agents derived from natural (ie, plant and animal) sources, consideration will be given only to acacia, gelatin, lecithin, and cholesterol. Many other natural materials are only sufficiently active to function as auxiliary emulsifying agents or stabilizers.

Acacia is a carbohydrate gum that is soluble in water and forms O/W emulsions. Emulsions prepared with acacia are stable over a wide pH range. Because it is a carbohydrate it is necessary to preserve acacia emulsions against microbial attack by the use of a suitable preservative. The gum can be precipitated from aqueous solution by the addition of high concentrations of electrolytes or solvents less polar than water, such as alcohol.

Gelatin, a protein, has been used for many years as an emulsifying agent. Gelatin can have two isoelectric points, depending on the method of preparation. So-called Type A gelatin, derived from an acid-treated precursor, has an isoelectric point of between pH 7 and 9. Type B gelatin, obtained from an alkali-treated precursor, has an isoelectric point of approximately pH 5. Type A gelatin acts best as an emulsifier around pH 3, where it is positively charged; on the other hand, Type B gelatin is best used around pH 8, where it is negatively charged. The question as to whether the gelatin is positively or negatively charged is fundamental to the stability of the emulsion when other charged emulsifying agents are present. In order to avoid an incompatibility, all emulsifying agents should carry the same sign. Thus, if gums (such as tragacanth, acacia or agar) which are negatively charged are to be used with gelatin, Type B material should be used at an alkaline pH. Under these conditions the gelatin is similarly negatively charged.

Lecithin is a phospholipid which, because of its strongly hydrophilic nature, produces O/W emulsions. It is liable to microbial attack and tends to darken on storage.

Cholesterol is a major constituent of wool alcohols, obtained by the saponification and fractionation of wool fat. It is cholesterol that gives wool fat its capacity to absorb water and form a W/O emulsion.

Finely Dispersed Solids—This group of emulsifiers forms particulate films around the dispersed droplets and produces emulsions which, while coarse-grained, have considerable physical stability. It appears possible that any solid can act as an emulsifying agent of this type, provided it is reduced to a sufficiently fine powder. In practice the group of compounds used most frequently are the colloidal clays.

Several colloidal clays find application in pharmaceutical emulsions; the most frequently used are bentonite, a colloidal aluminum silicate, and Veegum (*Vanderbilt*), a colloidal magnesium aluminum silicate.

Bentonite is a white to gray, odorless, and tasteless powder that swells in the presence of water to form a translucent suspension with a pH of about 9. Depending on the sequence of mixing it is possible to prepare both O/W and W/O emulsions. When an O/W emulsion is desired, the bentonite is first dispersed in water and allowed to hydrate so as to form a magma. The oil phase is then added gradually with constant trituration. Since the aqueous phase is always in excess, the O/W emulsion type is favored. To prepare a W/O emulsion, the bentonite is first dispersed in oil; the water is then added gradually.

While Veegum is used as a solid particle emulsifying agent, it is employed most extensively as a stabilizer in cosmetic lotions and creams. Concentrations of less than 1% Veegum will stabilize an emulsion containing anionic or nonionic emulsifying agents.

Auxiliary Emulsifying Agents—Included under this heading are those compounds which are normally incapable themselves of forming stable emulsions. Their main value lies in their ability to function as thickening agents and thereby help stabilize the emulsion. Agents in common use are listed in Table XVIII.

Emulsifying Agents and Emulsion Type

For a molecule, ion, colloid, or particle to be active as an emulsifying agent, it must have some affinity for the interface between the dispersed phase and the dispersion medium. With the mono- and multilayer films the emulsifier is in solution and, therefore, must be soluble to some extent in one or both of the phases. At the same time it must not be overly soluble in either phase, otherwise it will remain in the bulk of that phase and not be adsorbed at the interface. This balanced affinity for the two phases also must be evident with finely divided solid particles used as emulsifying agents. If their affinity, as evidenced by the degree to which they are wetted, is either predominantly hydrophilic or hydrophobic, they will not function as effective wetting agents.

The great majority of the work on the relation between

Table XIX—Approximate HLB Values for a Number of Emulsifying Agents

Generic or chemical name	HLB
Sorbitan trioleate	1.8
Sorbitan tristearate	2.1
Propylene glycol monostearate	3.4
Sorbitan sesquioleate	3.7
Glycerol monostearate (non self-emulsifying)	3.8
Sorbitan monooleate	4.3
Propylene glycol monolaurate	4.5
Sorbitan monostearate	4.7
Glyceryl monostearate (self-emulsifying)	5.5
Sorbitan monopalmitate	6.7
Sorbitan monolaurate	8.6
Polyoxyethylene-4-lauryl ether	9.5
Polyethylene glycol 400 monostearate	11.6
Polyoxyethylene-4-sorbitan monolaurate	13.3
Polyoxyethylene-20-sorbitan monooleate	15.0
Polyoxyethylene-20-sorbitan monopalmitate	15.6
Polyoxyethylene-20-sorbitan monolaurate	16.7
Polyoxyethylene-40-stearate	16.9
Sodium oleate	18.0
Sodium lauryl sulfate	40.0

emulsifier and emulsion type has been concerned with surface-active agents that form interfacial monolayers. The present discussion, therefore, will concentrate on this class of agents.

Hydrophile–Lipophile Balance—As the emulsifier becomes more hydrophilic, its solubility in water increases and the formation of an O/W emulsion is favored. Conversely, W/O emulsions are favored with the more lipophilic emulsifiers. This led to the concept that the type of emulsion is related to the balance between hydrophilic and lipophilic solution tendencies of the surface-active emulsifying agent.

Griffin[59] developed a scale based on the balance between these two opposing tendencies. This so-called *HLB scale* is a numerical scale, extending from 1 to approximately 50. The more hydrophilic surfactants have high HLB numbers (in excess of 10), while surfactants with HLB numbers from 1 to 10 are considered to be lipophilic. Surfactants with a proper balance in their hydrophilic and lipophilic affinities are effective emulsifying agents since they concentrate at the oil/water interface. The relationship between HLB values and the application of the surface-active agent is shown in Table XV. Some commonly used emulsifiers and their HLB numbers are listed in Table XIX. The utility of the HLB system in rationalizing the choice of emulsifying agents when formulating an emulsion will be discussed in a later section.

Rate of Coalescence and Emulsion Type—Davies[56] indicated that the type of emulsion produced in systems prepared by shaking is controlled by the relative coalescence rates of oil droplets dispersed in the oil. Thus, when a mixture of oil and water is shaken together with an emulsifying agent, a multiple dispersion is produced initially which contains oil dispersed in water and water dispersed in oil (Fig 19-36). The type of the final emulsion which results depends on whether the water or the oil droplets coalesce more rapidly. If the O/W coalescence rate (Rate 1) is much greater than W/O coalescence rate (Rate 2), a W/O emulsion is formed since the dispersed water droplets are more stable than the dispersed oil droplets. Conversely, if Rate 2 is significantly faster than Rate 1, the final emulsion is an O/W dispersion because the oil droplets are more stable.

According to Davies, the rate at which oil globules coalesce when dispersed in water is given by the expression

$$\text{Rate 1} = C_1 e^{-W_1/RT} \quad (39)$$

The term C_1 is a collision factor which is directly proportional to the phase volume of the oil relative to the water, and is an inverse function of the viscosity of the continuous phase (water). W_1 defines an energy barrier made up of several contributing factors that must be overcome before coalescence can take place. First, it depends on the electrical potential of the dispersed oil droplets, since this affects repulsion. Second, with an O/W emulsion, the hydrated layer surrounding the polar portion of emulsifying agent must be broken down before coalescence can occur. This hydrated layer is probably around 10 Å thick with a consistency of butter. Finally, the total energy barrier depends on the fraction of the interface covered by the emulsifying agent.

Equation 40 describes the rate of coalescence of water globules dispersed in oil, namely

$$\text{Rate 2} = C_2 e^{-W_2/RT} \quad (40)$$

Here, the collision factor C_2 is a function of the water/oil phase volume ratio divided by the viscosity of the oil phase. The energy barrier W_2 is, as before, related to the fraction of the interface covered by the surface-active agent. Another contributing factor is the number of $—CH_2—$ groups in the emulsifying agent; the longer the alkyl chain of the emulsifier, the greater the gap that has to be bridged if one water droplet is to combine with a second drop.

Davies[56] showed that the HLB concept is related to the distribution characteristics of the emulsifying agent between the two immiscible phases. An emulsifier with an HLB of less than 7 will be preferentially soluble in the oil phase and will favor formation of a W/O emulsion. Surfactants with an HLB value in excess of 7 will be distributed in favor of the aqueous phase and will promote O/W emulsions.

Preparation of Emulsions

Several factors must be taken into account in the successful preparation and formulation of emulsified products. Usually, the type of emulsion (ie, O/W or W/O) is specified; if not, it probably will be implied from the anticipated use of the product. The formulator's attention is focused primarily on the selection of the emulsifying agent, or agents, necessary to achieve a satisfactory product. No incompatibilities should occur between the various emulsifiers and the several components commonly present in pharmaceutical emulsions. Finally, the product should be prepared in such a way as not to prejudice the formulation.

Selection of Emulsifying Agents

The selection of the emulsifying agent, or agents, is of prime importance in the successful formulation of an emulsion. In addition to its emulsifying properties, the pharmacist must ensure that the material chosen is nontoxic and that the taste, odor, and chemical stability are compatible with the product. Thus, an emulsifying agent which is entirely suitable for inclusion in a skin cream may be unacceptable in the formulation of an oral preparation due to its potential toxicity. This consideration is most important when formulating intravenous emulsions.

The HLB System—With the increasing number of available emulsifiers, particularly the nonionics, the selection of emulsifiers for a product was essentially a trial-and-error procedure. Fortunately, the work of Griffin[59,60] provided a logical means of selecting emulsifying agents. Griffin's method, based on the balance between the hydrophilic and lipophilic portions of the emulsifying agent, is now widely used and has come to be known as the *HLB system*. It is used most in the rational selection of combinations of non-

Table XX—Relationship between HLB Range and Surfactant Application

HLB range	Use
0–3	Antifoaming agents
4–6	W/O emulsifying agents
7–9	Wetting agents
8–18	O/W emulsifying agents
13–15	Detergents
10–18	Solubilizing agents

Table XXI—Required HLB Values for Some Common Emulsion Ingredients

Substance	W/O	O/W
Acid, stearic	...	17
Alcohol, cetyl	...	13
Lanolin, anhydrous	8	15
Oil, cottonseed	...	7.5
mineral oil, light	4	10–12
mineral oil, heavy	4	10.5
Wax, beeswax	5	10–16
microcrystalline	...	9.5
paraffin	...	9

ionic emulsifiers, and we shall limit our discussion accordingly.

As shown in Table XX, if an O/W emulsion is required, the formulator should use emulsifiers with an HLB in the range of 8–18. Emulsifiers with HLB values in the range of 4–6 are given consideration when a W/O emulsion is desired. Some typical examples are given in Table XIX.

Another factor is the presence or absence of any polarity in the material being emulsified, since this will affect the polarity required in the emulsifier. Again, as a result of extensive experimentation, Griffin evolved a series of "required HLB" values; ie, the HLB value required by a particular material if it is to be emulsified effectively. Some values for oils and related materials are contained in Table XXI. Naturally, the required HLB value differs depending on whether the final emulsion is O/W or W/O.

Fundamental to the utility of the HLB concept is the fact that the HLB values are algebraically additive. Thus, by using a low HLB surfactant with one having a high HLB it is possible to prepare blends having HLB values intermediate between those of the two individual emulsifiers. Naturally, one should not use emulsifiers that are incompatible. The following formula should serve as an example.

O/W Emulsion

Liquid petrolatum (Required HLB 10.5)	50 g
Emulsifying agents	5 g
Sorbitan monooleate (HLB 4.3)	
Polyoxyethylene 20 sorbitan monooleate (HLB 15.0)	
Water, qs	100 g

By simple algebra it can be shown that 4.5 parts by weight of sorbitan monooleate blended with 6.2 parts by weight of polyoxyethylene 20 sorbitan monooleate will result in a mixed emulsifying agent having the required HLB of 10.5. Since the formula calls for 5 g, the required weights are 2.1 g and 2.9 g, respectively. The oil-soluble sorbitan monooleate is dissolved in the oil and heated to 75°; the water-soluble polyoxyethylene 20 sorbitan monooleate is added to the aqueous phase which is heated to 70°. At this point the oil phase is mixed with the aqueous phase and the whole stirred continuously until cool.

The formulator is not restricted to these two agents to produce a blend with an HLB of 10.5. Table XXII shows the various proportions required, using other pairs of emulsifying agents, to form a blend of HLB 10.5. When carrying out preliminary investigations with a particular material to be emulsified, it is advisable to try several pairs of emulsifying agents. Based on an evaluation of the emulsions produced, it becomes possible to choose the best combination.

Table XXII—Nonionic Blends having HLB Values of 10.5

Surfactant blend	HLB	Required amounts (%) to give HLB = 10 5
Sorbitan tristearate	2.1	34.4
Polyoxethylene 20 sorbitan monostearate	14.9	65.6
Sorbitan monopalmitate	6.7	57.3
Polyoxyethylene 20 sorbitan monopalmitate	15.6	42.7
Sorbitan sesquioleate	3.7	48.5
Polyoxyethylene lauryl ether	16.9	51.5

Occasionally, the required HLB of the oil may not be known, in which case it becomes necessary to determine this parameter. Various blends are prepared to give a wide range of HLB mixtures and emulsions are prepared in a standardized manner. The HLB of the blend used to emulsify the best product, selected on the basis of physical stability, is taken to be the required HLB of the oil. The experiment should be repeated using another combination of emulsifiers to confirm the value of the required HLB of the oil to within, say, ±1 HLB unit.

There are methods for finding the HLB value of a new surface-active agent. Griffin[60] developed simple equations which can be used to obtain an estimate with certain compounds. It has been shown that the ability of a compound to spread at a surface is related to its HLB. In another approach a linear relation between HLB and the logarithm of the dielectric constant for a number of nonionic surfactants has been observed. An interesting approach has been developed by Davies[56] and is related to his studies on the relative rates of coalescence of O/W and W/O emulsions (page 304). According to Davies, hydrophilic groups on the surfactant molecule make a positive contribution to the HLB number, whereas lipophilic groups exert a negative effect. Davies calculated these contributions and termed them HLB Group Numbers (Table XXIII). Provided the molecular structure of the surfactant is known, one simply adds the various group numbers in accordance with the following formula:

Table XXIII—HLB Group Numbers[61]

	Group number
Hydrophilic groups	
$-SO_4^-Na^+$	38.7
$-COO^-K^+$	21.1
$-COO^-Na^+$	19.1
N (tertiary amine)	9.4
Ester (sorbitan ring)	6.8
Ester (free)	2.4
$-COOH$	2.1
Hydroxyl (free)	1.9
$-O-$	1.3
Hydroxyl (sorbitan ring)	0.5
Lipophilic groups	
$-CH-$	
$-CH_2-$	
CH_3-	−0.475
$=CH-$	
Derived groups	
$-(CH_2-CH_2-O)-$	+0.33
$-(CH_2-CH_2-CH_2-O)-$	−0.15

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$$HLB = \Sigma(\text{hydrophilic group numbers}) - m(\text{group number}/\text{—}CH_2\text{— group}) + 7$$

where m is the number of $—CH_2—$ groups present in the surfactant. Poor agreement is found between the HLB values calculated by the use of group numbers and the HLB values obtained using the simple equations developed by Griffin. However, the student should realise that the absolute HLB values *per se* are of limited significance. The utility of the HLB approach (using values calculated by either Griffin's or Davies' equations) is to (i) provide the formulator with an idea of the relative balance of hydrophilicity and lipophilicity in a particular surfactant, and (ii) relate that surfactant's emulsifying and solubilizing properties to other surfactants. The formulator still needs to confirm experimentally that a particular formulation will produce a stable emulsion.

Later, Davies and Rideal[61] attempted to relate HLB to the C_{water}/C_{oil} partition coefficient and found good agreement for a series of sorbitan surfactants. Schott[62] showed, however, that the method does not apply to polyoxyethylated octylphenol surfactants. Schott concluded that "so far, the search for a universal correlation between HLB and another property of the surfactant which could be determined more readily than HLB has not been successful."

The HLB system gives no information as to the *amount* of emulsifier required. Having once determined the correct blend, the formulator must prepare another series of emulsions, all at the same HLB, but containing increasing concentrations of the emulsifier blend. Usually, the minimum concentration giving the desired degree of physical stability is chosen.

Mixed Emulsifying Agents—Emulsifying agents are frequently used in combination since a better emulsion usually is obtained. This enhancement may be due to several reasons, one or more of which may be operative in any one system. Thus, the use of a blend or mixture of emulsifiers may (1) produce the required hydrophile–lipophile balance in the emulsifier, (2) enhance the stability and cohesiveness of the interfacial film, and (3) affect the consistency and feel of the product.

The first point has been considered in detail in the previous discussion of the HLB system.

With regard to the second point, Schulman and Cockbain in 1940 showed that combinations of certain amphiphiles formed stable films at the air/water interface. It was postulated that the complex formed by these two materials (one, oil-soluble; the other, water-soluble) at the air/water interface was also present at the O/W interface. This interfacial complex was held to be responsible for the improved stability. For example, sodium cetyl sulfate, a moderately good O/W emulsifier, and elaidyl alcohol or cholesterol, both stabilizers for W/O emulsions, show evidence of an interaction at the air/water interface. Furthermore, an O/W emulsion prepared with sodium cetyl sulfate and elaidyl alcohol is much more stable than an emulsion prepared with sodium cetyl sulfate alone.

Elaidyl alcohol is the *trans* isomer. When oleyl alcohol, the *cis* isomer, is used with sodium cetyl sulfate, there is no evidence of complex formation at the air/water interface. Significantly, this combination does not produce a stable O/W emulsion either. Such a finding strongly suggests that a high degree of molecular alignment is necessary at the O/W interface to form a stable emulsion.

Finally, some materials are added primarily to increase the consistency of the emulsion. This may be done to increase stability or improve emolliency and feel. Examples include cetyl alcohol, stearic acid and beeswax.

When using combinations of emulsifiers, care must be taken to ensure their compatibility, as charged emulsifying agents of opposite sign are likely to interact and coagulate when mixed.

Small-Scale Preparation

Mortar and Pestle—This approach invariably is used only for those emulsions that are stabilized by the presence of a multimolecular film (eg, acacia, tragacanth, agar, chondrus) at the interface. There are two basic methods for preparing emulsions with the mortar and pestle. These are the *Wet Gum* (or so-called *English*) *Method* and the *Dry Gum* (or so-called *Continental*) *Method*.

The Wet Gum Method—In this method the emulsifying agent is placed in the mortar and dispersed in water to form a mucilage. The oil is added in small amounts with continuous trituration, each portion of the oil being emulsified before adding the next increment. Acacia is the most frequently used emulsifying agent when preparing emulsions with the mortar and pestle. When emulsifying a fixed oil, the optimum ratio of oil : water : acacia to prepare the initial emulsion is 4 : 2 : 1. Thus, the preparation of 60 mL of a 40% cod liver oil emulsion requires the following:

Cod liver oil	24 g
Acacia	6 g
Water, qs	60 mL

The acacia mucilage is formed by adding 12 mL of water to the 6 g of acacia in the mortar and triturating. The 24 g of oil is added in increments of 1–2 g and dispersed. The product at this stage is known as the *primary emulsion*, or *nucleus*. The primary emulsion should be triturated for at least 5 min, after which sufficient water is added to produce a final volume of 60 mL.

The Dry Gum Method—In this method, preferred by most pharmacists, the gum is added to the oil, rather than the water as with the wet gum method. Again, the approach is to prepare a primary emulsion from which the final product can be obtained by dilution with the continuous phase. If the emulsifier is acacia and a fixed oil is to be emulsified, the ratio of oil : water : gum is again 4 : 2 : 1.

Provided dispersion of the acacia in the oil is adequate, the dry gum method can almost be guaranteed to produce an acceptable emulsion. Because there is no incremental addition of one of the components, the preparation of an emulsion by this method is rapid.

With both methods the oil : water : gum ratio may vary, depending on the type of oil to be emulsified and the emulsifying agent used. The usual ratios for tragacanth and acacia are shown in Table XXIV.

The preparation of emulsions by both the wet and dry gum methods can be carried out in a bottle rather than a mortar and pestle.

Other Methods—An increasing number of emulsions are being formulated with synthetic emulsifying agents, especially of the nonionic type. The components in such a for-

Table XXIV—Usual Ratios of Oil, Water and Gum Used to Produce Emulsions

System	Acacia	Tragacanth
Fixed oils (excluding liquid petrolatum and linseed oil)	4	40
Water	2	20
Gum	1	1
Volatile oils, plus liquid petrolatum and linseed oil	2–3	20–30
Water	2	20
Gum	1	1

mulation are separated into those that are oil-soluble and those that are water-soluble. These are dissolved in their respective solvents by heating to about 70 to 75°. When solution is complete, the two phases are mixed and the product is stirred until cool. This method, which requires nothing more than two beakers, a thermometer and a source of heat, is necessarily used in the preparation of emulsions containing waxes and other high-melting-point materials that must be melted before they can be dispersed in the emulsion. The relatively simple methodology involved in the use of synthetic surfactant-type emulsifiers is one factor which has led to their widespread use in emulsion preparation. This, in turn, has led to a decline in the use of the natural emulsifying agents.

With hand homogenizers an initial rough emulsion is formed by trituration in a mortar or shaking in a bottle. The rough emulsion is then passed several times through the homogenizer. A reduction in particle size is achieved as the material is forced through a narrow aperture under pressure. A satisfactory product invariably results from the use of a hand homogenizer and overcomes any deficiencies in technique. Should the homogenizer fail to produce an adequate product, the formulation, rather than the technique, should be suspected.

For a discussion of the techniques and equipment used in the large-scale manufacture of emulsions, see Chapter 83.

Stability of Emulsions

There are several criteria which must be met in a well-formulated emulsion. Probably the most important and most readily apparent requirement is that the emulsion possess adequate physical stability; without this, any emulsion soon will revert back to two separate bulk phases. In addition, if the emulsified product is to have some antimicrobial activity (eg, a medicated lotion), care must be taken to ensure that the formulation possesses the required degree of activity. Frequently, a compound exhibits a lower antimicrobial activity in an emulsion than, say, in a solution. Generally, this is because of partitioning effects between the oil and water phases, which cause a lowering of the "effective" concentration of the active agent. Partitioning has also to be taken into account when considering preservatives to prevent microbiological spoilage of emulsions. Finally, the chemical stability of the various components of the emulsion should receive some attention, since such materials may be more prone to degradation in the emulsified state than when they exist as a bulk phase.

In the present discussion, detailed consideration will be limited to the question of physical stability. Reviews of this topic have been published by Garrett[63] and Kitchener and Mussellwhite.[64] For information on the effect that emulsification can have on the biologic activity and chemical stability of materials in emulsions, see Wedderburn,[65] Burt[66] and Swarbrick.[67]

The theories of emulsion stability have been discussed by Eccleston[68] in an attempt to understand the situation in both a simple O/W emulsion and complex commercial systems.

The three major phenomena associated with physical stability are

1. The upward or downward movement of dispersed droplets relative to the continuous phase, termed *creaming* or *sedimentation*, respectively.
2. The aggregation and possible coalescence of the dispersed droplets to reform the separate, bulk phases.
3. Inversion, in which an O/W emulsion inverts to become a W/O emulsion, and *vice versa*.

Creaming and Sedimentation—Creaming is the upward movement of dispersed droplets relative to the continuous phase, while sedimentation, the reverse process, is the downward movement of particles. In any emulsion one process or the other takes place, depending on the densities of the disperse and continuous phases. This is undesirable in a pharmaceutical product where homogeneity is essential for the administration of the correct and uniform dose. Furthermore, creaming, or sedimentation, brings the particles closer together and may facilitate the more serious problem of coalescence.

The rate at which a spherical droplet or particle sediments in a liquid is governed by Stokes' law (Eq 35). While other equations have been developed for bulk systems, Stokes' equation is still useful since it points out the factors that influence the rate of sedimentation or creaming. These are the diameter of the suspended droplets, the viscosity of the suspending medium, and the difference in densities between the dispersed phase and the dispersion medium.

Usually, only the use of the first two factors is feasible in affecting creaming or sedimentation. Reduction of particle size contributes greatly toward overcoming or minimizing creaming, since the rate of movement is a square-root function of the particle diameter. There are, however, technical difficulties in reducing the diameter of droplets to below about 0.1 μm. The most frequently used approach is to raise the viscosity of the continuous phase, although this can be done only to the extent that the emulsion still can be removed readily from its container and spread or administered conveniently.

Aggregation and Coalescence—Even though creaming and sedimentation are undesirable, they do not necessarily result in the breakdown of the emulsion, since the dispersed droplets retain their individuality. Furthermore, the droplets can be redispersed with mild agitation. More serious to the stability of an emulsion are the processes of aggregation and coalescence. In aggregation (flocculation) the dispersed droplets come together but do not fuse. Coalescence, the complete fusion of droplets, leads to a decrease in the number of droplets and the ultimate separation of the two immiscible phases. Aggregation precedes coalescence in emulsions; however, coalescence does not necessarily follow from aggregation. Aggregation is, to some extent, reversible. While not as serious as coalescence, it will accelerate creaming or sedimentation, since the aggregate behaves as a single drop.

While aggregation is related to the electrical potential on the droplets, coalescence depends on the structural properties of the interfacial film. In an emulsion stabilized with surfactant-type emulsifiers forming monomolecular films, coalescence is opposed by the elasticity and cohesiveness of the films sandwiched between the two droplets. In spite of the fact that two droplets may be touching, they will not fuse until the interposed films thin out and eventually rupture. Multilayer and solid-particle films confer on the emulsion a high degree of resistance to coalescence, due to their mechanical strength.

Particle-size analysis can reveal the tendency of an emulsion to aggregate and coalesce long before any visible signs of instability are apparent. The methods available have been reviewed by Groves and Freshwater.[69]

Inversion—An emulsion is said to invert when it changes from an O/W to a W/O emulsion, or *vice versa*. Inversion sometimes can be brought about by the addition of an electrolyte or by changing the phase–volume ratio. For example, an O/W emulsion having sodium stearate as the emulsifier can be inverted by the addition of calcium chloride, because the calcium stearate formed is a lipophilic emulsifier and favors the formation of a W/O product.

Inversion often can be seen when an emulsion, prepared by heating and mixing the two phases, is being cooled. This takes place presumably because of the temperature-depen-

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dent changes in the solubilities of the emulsifying agents. The phase inversion temperature, or PIT, of nonionic surfactants has been shown by Shinoda, *et al*[70] to be influenced by the HLB number of the surfactant. The higher the PIT value, the greater the resistance to inversion.

Apart from work on PIT values, little quantitative work has been carried out on the process of inversion; nevertheless, it would appear that the effect can be minimized by using the proper emulsifying agent in an adequate concentration. Wherever possible, the volume of the dispersed phase should not exceed 50% of the total volume of the emulsion.

Bioavailability from Coarse Dispersions

In recent years, considerable interest has focused on the ability of a dosage form to release drug following administration to the patient. Both the rate and extent of release are important. Ideally, the extent of release should approach 100%, while the rate of release should reflect the desired properties of the dosage form. For example, with products designed to have a rapid onset of activity, the release of drug should be immediate. With a long-acting product, the release should take place over several hours, or days, depending on the type of product used. The rate and extent of drug release should be reproducible from batch to batch of the product, and should not change during shelf life.

The principles on which biopharmaceutics is based are dealt with in some detail in Chapters 35 to 37. While most published work in this area has been concerned with the bioavailability of solid dosage forms administered by the oral route, the rate and extent of release from both suspensions and emulsions is important and so will be considered in some detail.

Bioavailability from Suspensions—Suspensions of a drug may be expected to demonstrate improved bioavailability compared to the same drug formulated as a tablet or capsule. This is because the suspension already contains discrete drug particles, whereas tablet dosage forms must invariably undergo disintegration in order to maximize the necessary dissolution process. Frequently, antacid suspensions are perceived as being more rapid in action and therefore more effective than an equivalent dose in the form of tablets. Bates, *et al*[71] observed that a suspension of salicylamide was more rapidly bioavailable, at least during the first hour following administration, than two different tablet forms of the drug; these workers were also able to demonstrate a correlation between the initial *in vitro* dissolution rates for the several dosage forms studied and the initial rates of *in vivo* absorption. A similar argument can be developed for hard gelatin capsules, where the shell must rupture or dissolve before drug particles are released and can begin the dissolution process. Such was observed by Antal, *et al*[72] in a study of the bioavailability of several doxycycline products, including a suspension and hard gelatin capsules. Sansom, *et al*[73] found mean plasma phenytoin levels higher after the administration of a suspension than when an equivalent dose was given as either tablets or capsules. It was suggested that this might have been due to the suspension having a smaller particle size.

In common with other products in which the drug is present in the form of solid particles, the rate of dissolution and thus potentially the bioavailability of the drug in a suspension can be affected by such factors as particle size and shape, surface characteristics, and polymorphism. Strum, *et al*[74] conducted a comparative bioavailability study involving two commercial brands of sulfamethiazole suspension (Product A and Product B). Following administration of the products to 12 normal subjects and taking blood samples at predetermined times over a period of 10 hr, the workers found no statistically significant difference in the extent of drug absorption from the two suspensions. The absorption rate, however, differed, and from *in vitro* studies it was concluded that product A dissolved faster than product B and that the former contained more particles of smaller size than the latter, differences that may be responsible for the more rapid dissolution of particles in product A. Product A also provided higher serum levels in *in vivo* tests half an hour after administration. The results showed that the rate of absorption of sulfamethiazole from a suspension depended on the rate of dissolution of the suspended particles, which in turn was related to particle size. Previous studies[75,76] have shown the need to determine the dissolution rate of suspensions in order to gain information as to the bioavailability of drugs from this type of dosage form.

The viscosity of the vehicle used to suspend the particles has been found to have an effect on the rate of absorption of nitrofurantoin but not the total bioavailability. Thus Soci and Parrott were able to maintain a clinically acceptable urinary nitrofurantoin concentration for an additional two hours by increasing the viscosity of the vehicle.[77]

Bioavailability from Emulsions—There are indications that improved bioavailability may result when a poorly absorbed drug is formulated as an orally administered emulsion. However, little study appears to have been made in direct comparison of emulsions and other dosage forms such as suspensions, tablets, and capsules; thus it is not possible to draw unequivocal conclusions as to advantages of emulsions. If a drug with low aqueous solubility can be formulated so as to be in solution in the oil phase of an emulsion, its bioavailability may be enhanced. It must be recognized, however, that the drug in such a system has several barriers to pass before it arrives at the mucosal surface of the gastrointestinal tract. For example, with an oil-in-water emulsion, the drug must diffuse through the oil globule and then pass across the oil/water interface. This may be a difficult process, depending on the characteristics of the interfacial film formed by the emulsifying agent. In spite of this potential drawback, Wagner, *et al*[78] found that indoxole, a nonsteroidal anti-inflammatory agent, was significantly more bioavailable in an oil-in-water emulsion than in either a suspension or a hard gelatin capsule. Bates and Sequeira[79] found significant increases in maximum plasma levels and total bioavailability of micronized griseofulvin when formulated in a corn oil/water emulsion. In this case, however, the enhanced effect was not due to emulsification of the drug in the oil phase *per se* but more probably because of the linoleic and oleic acids present having a specifical effect on gastrointestinal motility.

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CHAPTER 28

Clinical Analysis

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The characterization and quantitation of the various components of blood, urine and other body fluids are the primary functions of the clinical laboratory. The major divisions of clinical analysis are clinical biochemistry, hematology, blood-bank technology, histopathology, immunology and microbiology. The accurate diagnosis of disease and determination of a potential therapeutic regimen frequently are based on the laboratory analysis of blood, urine, feces, gastric secretions or cerebrospinal fluid. Modern medical practice is tending toward greater reliance on laboratory results as definitive measures of pathological or normal states.

The pharmacist should familiarize himself with the basic principles involved in sample collection, analysis and diagnostic significance of the various clinical parameters. His role in community health necessitates his comprehension of the methodology and diagnostic value of clinical laboratory procedures. The influence of various drugs and drug interactions on these parameters must be considered in both the clinical and drug-abuse situation.

Hematology

The determination of the morphological, physiological and biochemical properties of peripheral blood and the blood-forming organs (hematopoietic system) is a function of the hematology laboratory. The functional categories of hematology are (1) analysis of cellular elements, and specific biochemical and physiological parameters of peripheral blood and the hematopoietic system, (2) blood-coagulation analysis and (3) blood-bank technology.

Peripheral blood is a biphasic liquid tissue system of cellular elements suspended in a liquid plasma phase. The cellular phase comprises about 45% of the blood volume and contains erythrocytes (red blood cells, RBC), leukocytes (white blood cells, WBC) and thrombocytes (platelets). The plasma phase is primarily water (90 to 92%) and protein (7%).

The hematological analysis of blood is concerned primarily with enumeration and differentiation of the various cellular elements. An analysis of the hematopoietic system (eg, bone marrow and lymphoid tissue) determines the status of blood-cell precursors in these tissues. Determinations of specific biochemical (hemoglobin) and physiological (blood or plasma volume) parameters are performed in a complete evaluation of the erythron system (blood and marrow RBC and their precursors). The normal hematological values in the adult are presented in Table I.

The authors acknowledge the assistance of Dr Joseph P Uscavage of Rorer Group Ltd, in the preparation of the *Microbiology* section and Dr Alfred H Free of the Ames Co for the *Urinalysis* section.

Erythrocytes and Hemoglobin—The erythrocytic system is composed of the mature erythrocytes in peripheral blood and their precursors in bone marrow. The precursors of erythrocytes, as found in the erythropoietic system (red bone marrow), are classified as to the degree of nucleation and characteristics of cytoplasmic constituents. The sequence of erythrocyte formation in bone marrow—based on the gradual denucleation of the cell, generation of the chromatin structure and changes in nucleolar structure and cytoplasmic constituents—is as follows:

pronormoblast → basophilic normoblast → polychromatic normoblast → orthochromatic normoblast → polychromatophilic erythrocyte → erythrocyte.

The first four types are nucleated and normally are seen only in bone marrow. In normal erythrocyte formation these immature bone-marrow cells are designated as *normoblastic* or *normocytic*. In pernicious anemia and related conditions they become abnormally large and are designated *megaloblastic* or *megalocytic*. In iron-deficiency anemia, these cells become abnormally small and are designated *microblastic* or *microcytic*—of the iron-deficiency type.

Table I—Normal Hematological Values in Man[1]

	Normal Value	Normal Range of Values
Erythrocytes (cu mm × 10^6)		
Male	5.4	4.6–6.2
Female	4.8	4.2–5.6
Reticulocytes (cu mm × 10^3)	50	10–100
Hemoglobin (g%)		
Male	16.0	14.0–18.0
Female	14.0	12.0–16.0
Hematocrit (%)		
Male	47.0	40.0–54.0
Female	42.0	37.0–47.0
Mean corpuscular volume (μm^3)	87	82–92
Mean corpuscular hemoglobin (pg)	29	27–31
Mean corpuscular hemoglobin concentration (%)	34	32–36
Mean corpuscular diameter (μm)	7.3	6.7–7.7
Leukocytes (cu mm × 10^3)	7.0	5.0–10.0
Leukocyte differential (%)		
Neutrophils	63	57–67
Eosinophils	1	1–3
Basophils	1	0–1
Lymphocytes	30	25–33
Monocytes	5	3–7
Platelets (cu mm × 10^5)	3.0	1.4–6.0
Erthrocyte sedimentation rate (Wintrobe), (mm/hr)		
Male	4	0–9
Female	10	0–20

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Normal blood contains 0.5 to 1.5% of circulating erythrocytes as reticulocytes. These cells contain a fine network of basophilic reticulum that is demonstrable on staining with a vital dye such as brilliant cresyl blue. The number of these cells in the blood is a measure of effective erythropoiesis. High circulating-reticulocyte values are an index of erythropoietic activity and are found in the first few days of life, after hemorrhage and after treatment of iron- or vitamin B_{12}-deficiency anemias.

The normal *erythrocyte* (normocyte) is a flexible, elastic, biconcave, enucleated structure with a mean diameter of 7.3 μm and a thickness near 2.2 μm. The chemical constituents of the red blood cell include water (63%), lipids (0.5%), glucose (0.8%), minerals (0.7%), nonhemoglobin protein (0.9%), methemoglobin (0.5%) and hemoglobin (33.6%). The primary function of the erythrocyte is transport of oxygen and carbon dioxide. The red cell membrane, a dynamic, semipermeable component of the cell, is associated with energy metabolism in the maintenance of the permeability characteristics of the cell to various cations (Na^+, K^+) and anions (Cl^-, HCO_3^-). The stroma of insoluble material which remains after red-cell disruption (hemolysis) constitutes 2 to 5% of the wet-cell weight; it is primarily protein (40 to 60%) and lipid (10 to 12%). The membrane includes stromatin (a fibrous or structural protein) and mucopolysaccharides associated with A, B and O blood-group substances. The lipid fractions include phosphatides (lecithin, cephalin), cholesterol, cholesterol esters, neutral fats, cerebrosides and sialic acid glycoproteins.

Erythrocytes may be enumerated by either visual or electronic procedures. In the visual procedures, a measured quantity of blood is diluted with a fluid which is isotonic with blood and will prevent its coagulation. The diluted blood is then placed in a counting chamber (hemocytometer), and the number of cells in a circumscribed area is enumerated microscopically. Hayem's solution (sodium sulfate, 2.5 g; sodium chloride, 0.25 g; mercuric chloride, 0.25 g; distilled water, 100 mL), Toison's fluid (sodium sulfate, 8 g; sodium chloride, 1 g; methyl violet, 0.025 g; glycerin, 30 mL; distilled water, 180 mL) or 0.9% sodium chloride are used as diluting fluids. The overall error of this method is about 8%.

A greater degree of accuracy and reproducibility can be achieved by erythrocyte enumeration in an electronic counting apparatus; eg, Coulter Counter or Ortho cell counters. The Coulter method (Fig 28-1) determines the number and size of particles suspended in an electrically conductive liquid. The blood cells traverse a small aperture and displace their own volume in the diluent as to produce a change in resistance between the electrodes; the magnitude of the voltage pulse is proportional to cell volume, and the resultant pulses are then amplified, scaled and automatically counted.

In the Ortho ELT-8 technique (Fig 28-2), the principles of laser flow cytometry are used to count cells. Hydrodynamic focusing and laminar flow are combined in the system to count a large number of individual cells. Light focused by a helium-neon laser is scattered by the cells as they pass through the flow channel. The scattered light is monitored by a photoelectric sensor and transfers the electrical pulses which are processed by the systems circuitry. In addition to increased counting speed, the overall error of the electronic procedures is reduced to about 1%.

The *hematocrit value* is also a measure of the erythrocyte portion of blood. A sample of blood containing an anticoagulant is placed in a graduated hematocrit capillary tube, centrifuged and the volume ratio of packed red cells to total blood volume (hematocrit value) determined. The centrifuged sample appears as a red layer of packed erythrocytes over which is found an off-white layer of packed leukocytes and platelets, and a supernatant plasma phase. The hematocrit value is an index of both the number and size of the red cells.

Hemoglobin, a conjugated hemoprotein with an approximate molecular weight of 67,000, contains basic proteins, the globins and ferroprotoporphyrin (heme). It is essentially a tetramer, consisting of four peptide chains, to each of which is bound a heme group. Heme, which constitutes about 4% of the weight of the molecule, consists of a divalent iron atom in the center of a pyrrole-porphyrin structure. Four distinct polypeptide chains (α, β, γ, δ) can be incorporated into hemoglobin. Normal adult hemoglobin is HbA = $\alpha_2{}^A\beta_2{}^A$. Fetal hemoglobin contains 2α and 2γ chains and is designated HbF = $\alpha_2{}^A\gamma_2{}^F$.

Differences in the structural sequences of amino acids in the peptide portion of the hemoglobin molecules are controlled genetically and are responsible for different types of hemoglobin. Based on the characteristic mobility of the hemoglobin, in an electric field (electrophoresis) on starch, paper, cellulose acetate, agar or acrylamide gel media, many hemoglobin types have been recognized (see Chapter 29). Only types P, F and A_1-A_4 are considered normal. Sickle-cell anemia and β-thalassemia are hemolytic anemias associated with abnormal hemoglobins (ie, Type S in sickle-cell anemia and abnormal production of the β chain in β-thalas-

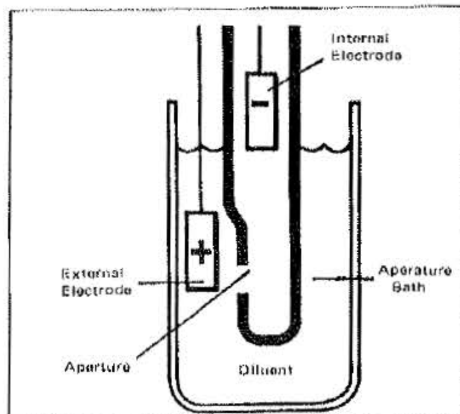

Fig 28-1. Coulter-counting cells by electronic impedance (courtesy Coulter Electronics).

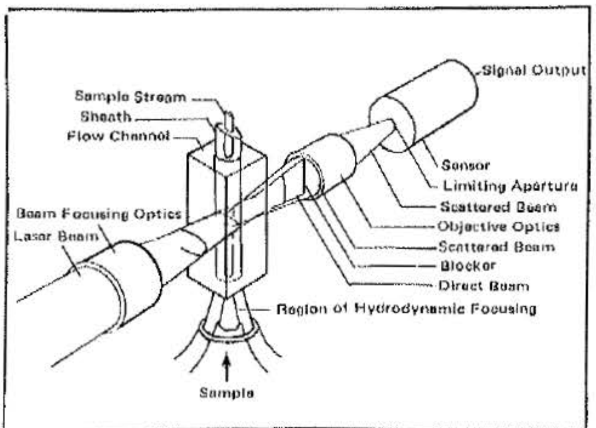

Fig 28-2. Ortho ELT-8-Method of scattered light detection and hydrodynamic focusing for cell counting (courtesy, Clinical Instrument Systems, Oct 1980).

semia). In homozygous *HbS disease* sickling of the red cells is due to the low solubility of the abnormal hemoglobin in its reduced state, with the production of semicrystalline bodies (tactoids), which distort and elongate the cells. In the sickle-cell trait (heterozygous), the blood smear shows no sickle cells. In the homozygous condition, HbS accounts for nearly all of the hemoglobin with small amounts of HbF. In the heterozygous condition, HbS constitutes 50% or less of the hemoglobin, with the balance as HbA.

The detection of sickle-cell disease is performed by microscopic observation of the induction of red-cell sickling in the presence of a reducing agent such as sodium metabisulfite or by quantitative determination of urea-dispersible turbidity induced by dithionite following reduction of HbS to deoxy-HbS in RBC lysates. The microscopic procedure will detect only homozygotes, whereas HbAS and HbS and its structural variant HbC-Harlem both are detected in the urea-dithionite technique. Commercial qualitative test kits are available for detecting sickle-cell trait and anemia by solubility determinations. All hemoglobins positive to the dithionite test must be electrophorized (cellulose acetate, citrate agar or starch gel) to differentiate HbS from HbC and thalassemia traits. Drugs causing hemolysis in glucose 6-phosphate dehydrogenase (G6PD) deficiency include sulfones, nitrofurans, chloroquine, dimercaprol, nalidixic acid and probenecid.

The *hemoglobin* concentration is measured spectrophotometrically after lysis of whole blood and conversion of hemoglobin to hematin, oxyhemoglobin or cyanmethemoglobin. The addition of a strong base (NaOH) to pH 10 converts oxyhemoglobin, carboxyhemoglobin and methemoglobin to hematin, which can be estimated photometrically. Weaker bases (Na_2CO_3 or NH_4OH) convert hemoglobin to oxyhemoglobin for analysis.

Total hemoglobin is measured also by conversion to cyanmethemoglobin using alkaline sodium cyanide-potassium ferricyanide reagent. Hemoglobin standards certified by the Clinical Standards Committee of the College of American Pathologists are used in these procedures, and all results are expressed as "g hemoglobin per 100 mL blood."

In the normal state, the oxygen consumption of the RBC is low and it is involved in the conversion of hemoglobin to oxidized (Fe^{3+}) methemoglobin (HbM) which cannot bind oxygen. The normal balance of HbM (<0.5%) is maintained by two enzyme systems—NADH and NADPH methemoglobin reductases. An inherited deficiency of the RBC enzyme, G6PD. This will decrease the rate of reduction of glutathione and methemoglobin, make the cell more vulnerable to oxidative attack and result in susceptibility to drug-induced or immune-mediated nonspherocytic hemolytic anemia. G6PD deficiency is found predominantly in Mediterranean peoples, Southeast Asians, Africans and American negroes. The enzyme can be quantitated spectrometrically or by fluoronephelometry by measuring the rate of reduction of nicotinamide adenine dinucleotide phosphate (NADP) in the presence of G6PD. Presumptive screening tests based on reduced glutathione (GSH) content of blood before and after incubation with acetylphenylhydrazine also are used.

Erythrocyte count, hemoglobin content and hematocrit value are used to determine various blood indices in the diagnosis and treatment of anemia. These measurements are:

$$\textit{Mean corpuscular volume } [\text{MCV } (\mu m^3)] = \frac{\text{Hematocrit } (\%) \times 10}{\text{Erythrocyte count (millions/cu mm)}}$$

$$\textit{Mean corpuscular hemoglobin } [\text{MCH } (pg)] = \frac{\text{Hemoglobin } (g/100 \text{ mL}) \times 10}{\text{Erythrocyte count (millions/cu mm)}}$$

$$\textit{Mean corpuscular hemoglobin concentration } [\text{MCHC}(\%)] = \frac{\text{Hemoglobin } (g/100 \text{ mL}) \times 100}{\text{Hematocrit } (\%)}$$

An additional parameter used to characterize red-cell variation is the red-cell distribution width (RDW) determined on the Coulter S-Plus II. The RDW is calculated directly by the standard deviation and coefficient of variation from a red-cell histogram on the S-Plus II. The difference in cell size may be used to monitor patients with pernicious or hemorrhagic anemia.

Anemias are classified as to red-cell volume and hemoglobin concentration. *Macrocytic* (large cell: MCV > 94), *normocytic* (normal cell: MCV, 82 to 92), or *microcytic* (small cell: MCV < 80) are the classifications according to cell volume. Cellular hemoglobin concentration categorizes the cells as to *hyperchromic* (MCHC > 38), *normochromic* (MCHC = 32 to 36), or *hypochromic* (MCHC < 30). Examples of anemias:

I. Hypochromic Microcytic—erythroid normoblastic anemia in bone marrow
   A. Iron Deficiency—low hemoglobin (Hbg) and RBC, low serum iron, high total iron binding capacity, absent hemosiderin.
      1. Dietary—low iron intake
      2. Intestinal problems—decreased iron absorption
      3. Pregnancy, infants—increased iron requirements
      4. Iron loss—due to chronic hemorrhage, parasitic infections, GI tract lesions, excess menstrual bleeding.
   B. Hereditary Sideroblastic—defect in the heme synthesis, an inability to utilize ingested iron.
   C. Thalassemia—genetic abnormality which produces normal to increased HbgF and/or HbgA_2.
II. Normochromic Normocytic
   A. Hemolytic—increased destruction of erythrocytes.
      1. Autoimmune hemolytic
      2. Cold agglutinin hemolytic
      3. Mechanical destruction of RBCs
      4. Paroxysmal Nocturnal hemoglobinuria
      5. Lymphomas and Hodgkin's disease
      6. Infections
   B. Hemoglobinopathies—abnormalities in structure of alpha or beta chains of hemoglobin molecule; normoblastic erythroidhyperplasia in bone marrow.
      1. Sickle-cell
      2. Hemolysis
      3. Hemoglobin CC
   C. Acute Hemorrhage
   D. Other
      1. Aplastic Anemia, Leukemia, Malignancy
      2. Renal failure and drug-related anemias caused by chloramphenicol and antineoplastic drugs.
III. Normochromic Macrocytic—due to deficiency of vitamin B_{12} or folate; bone marrow is hypercellular with increased erythroid precursors.
      1. Pernicious
      2. Sideroblastic
      3. Sprue—total iron-binding capacity is decreased; hemosiderin is increased in the bone marrow.
      4. Pregnancy

Determinations of the suspension stability of whole blood and erythrocyte fragility are useful adjuncts in the diagnosis of various diseases.

The *erythrocyte sedimentation rate* (ESR) is an estimate of the suspension stability of red blood cells in plasma; it is related to the number and size of the red cells and to the relative concentration of plasma proteins, especially fibrinogen and the α- and β-globulins. This test is performed by determining the rate of sedimentation of blood cells in a standard tube. Normal blood ESR is 0 to 15 mm/hour. Increases are an indication of active but obscure disease processes such as tuberculosis and ankylosing spondylitis. ESR is affected by anemia and does not respond linearly with changes in asymmetrical macromolecules such as fibrinogen and globins.

The *zeta sedimentation ratio* (ZSR) technique overcomes these disadvantages. It is based on a measure of the closeness with which RBC will

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approach each other after standardized cycles of dispersion and compaction.

The *erythrocyte fragility test* is based on resistance of cells to hemolysis in decreasing concentrations of hypotonic saline.

Increased osmotic fragility of the red cells is associated with various types of spherocytosis and acquired hemolytic anemia; increased resistance has been observed in thalassemia, sickle-cell anemia and hypochromic anemia. The test can be performed manually by colorimetric estimation of hemoglobin released by hypotonic cell rupture or automatically in an instrument which continually records the increase in light transmittance through a suspension of red cells in a continuously decreasing salt gradient during dialysis.

Leukocytes—Mature *leukocytes* (white blood cells, WBC) in peripheral blood and their precursors in bone and lymphoid tissue comprise the leukocytic system. Various types of leukocytes are found in normal blood. Differentiation of the lymphocytic, monocytic and granulocytic leukocyte types is based on cell size, color, chromatin structure and cytoplasm constituents.

The primary function of leukocytes is the development of the various defensive and reparative processes in inflammatory and immune-response mechanisms. The migration of leukocytes to the site of inflammation is associated with the release or activation of various biochemical substances (5-hydroxytryptamine, histamine, complement, immunoglobulins, prostaglandins, lysosomal enzymes). The tissue histiocyte or monocyte (macrophage) also can engulf and destroy foreign particles by the process of endocytosis and certain leukocyte types by phagocytosis.

The chemical composition of the leukocyte includes water (82%), nucleoprotein, phospholipids and trace minerals. Enzyme content, glycogen and histamine levels vary in the different types of white cells. Deficiency in enzymes associated with glycolytic metabolism (hexokinase) and increases in phosphomonoester hydrolases (alkaline phosphatase) have been observed in leukocytes of certain leukemia patients.

The precursors of granulocytic leukocytes are found in bone marrow and are classified according to the degree of cytoplasmic granulation, dye-affinity of the granules and shape of the nucleus (Schilling, Arneth or Cooke-Ponder Classification). As undifferentiated cells (myeloblasts) mature

promyelocyte → myelocyte → metamyelocyte → band leukocyte → segmented leukocyte

metachromatic granules appear in the cytoplasm (granulocytes). All segmented leukocytes are motile, a requirement for participation in the inflammatory or phagocytic processes.

In the mature *basophilic* and *eosinophilic leukocytes*, these granules develop an affinity for a basic or acidic dye, respectively; those cells containing granules which do not stain are called ***neutrophils.*** In peripheral blood, the mature granulocytic cells are designated *polymorphonuclear leukocytes—neutrophilic, eosinophilic or basophilic.*

The other types of white cells normally observed in peripheral blood have no granules and are classified as to size and shape into the *monocyte* and *lymphocyte*, which are formed in lymphoid tissue. The small lymphocyte is thymic-derived and is found in the circulation and germinal centers of lymphoid tissue. The origin of the large lymphocyte is a gut-associated lymphoid stem cell which can further differentiate into the immunoglobulin-producing plasmacyte. The interaction of thymic (T) and bone-marrow (B) lymphocytes is the basis for the development and maintenance of humoral and cellular immune mechanisms.

Leukocytes are enumerated by procedures similar to those used for erythrocytes. In the visual procedures the blood is diluted with a fluid (3% v/v acetic acid) which lyses the red cells, and the total leukocyte count is determined microscopically. Eosinophils also may be analyzed differentially with a diluting fluid which renders the red cells nonrefractile and invisible, and lyses the base-labile leukocytes, leaving the base-stable eosinophils intact. A suitable diluting fluid for this purpose is Pilot's Fluid (propylene glycol, 50 mL; distilled water, 40 mL; 1% phloxine, 10 mL; 10% sodium carbonate, 1 mL and heparin sodium, 100 units). Electronic-counting procedures are similar to those used for erythrocytes with the added advantages of speed, accuracy and reproducibility.

The normal adult leukocyte value is 5000 to 10,000 cells/cu mm. Values greater than 10,000 (*leukocytosis*) are encountered in the newborn infant, young children, after violent exercise, convulsive seizures of epilepsy, leukemia and cancer. Values of less than 5000 (*leukopenia*) are observed in certain microbial infections (eg, typhoid fever, measles, malaria, overwhelming septicemia), cirrhosis of the liver, pernicious anemia, radiation injury and replacement of marrow by malignant tissue.

A *differential count of the leukocytes* provides information as to the relative numbers of each type. A thin film of blood is prepared on a microscope slide stained with a polychromatic preparation such as the Leishman, Wright or Giemsa stain, and analyzed microscopically. Wright's stain contains polychromed methylene blue and eosin dyes; the erythrocytes are stained pink; the nuclei of the leukocytes, purplish-blue; neutrophilic granules, violet-pink; eosinophilic granules, red; basophilic granules, blue; and platelets, blue.

The recent introduction of automated systems for differential white-cell counts significantly reduce the errors inherent with the subjective nature of the visual counting procedure. Differentiation of the various cell types can be made on the basis of cytochemistry and staining properties of enzymes specific for a single cell type. The granules of neutrophils and eosinophils are stained by action of their peroxidases on 4-chloro-1-naphthol to form a colored quinone in the presence of a peroxide and further differentiated by the optimum pH for peroxidase activity between these two cell types. The monocytic lipase is used as a specific marker by the reaction of basic fuchsin with α-naphthol liberated by lipase on α-naphthylbutyrate substrate. The lymphocytes are not stained in this procedure but are measured by electronic sizing.

Automated differential WBC counts also have been obtained in systems which count large populations of cells by simultaneous measurement of two optical properties (axial light loss and/or narrow-angle scatter and/or multiple-wavelength fluorescence). Laser light also is used to differentiate cell size, granularity and volume of cells. The collected light measured by forward versus right-angle scatter is converted to a histogram giving the percent of lymphocytes, monocytes and granulocytes. Another system involves computer processing of two-dimensional images of the various cell types using an automatic scanning microscope.

Polymorphonuclear neutrophilic leukocytes (neutrophils, "polys") normally comprise 62% (50 to 67%) of the total leukocyte count. These cells are irregular in shape (10 to 15 μm in diameter) and usually contain a multilobated nucleus with fine, lightly stained cytoplasmic granules. An immature or juvenile form of neutrophil, with a band-shaped nonsegmented nucleus constitutes 3 to 5% of peripheral blood leukocytes. Increases in the relative percentage of these cells (neutrophilia) is observed in acute microbial infections (eg, meningitis, smallpox, poliomyelitis), metabolic disorders (diabetic acidosis, gout), drug intoxication (digitalis, epinephrine), vaccination, coronary thrombosis and malignant neoplasms.[2]

Polymorphonuclear eosinophilic leukocytes (eosinophils) normally comprise about 1 to 3% of total circulating white-blood cells. In appearance they are similar to the neutrophil with the exception of large, red-stained cytoplasmic granules. Eosinophilia has been observed in certain

skin diseases (psoriasis, eczema), parasitic infestations (pork round worm—trichinosis), certain hypersensitivity reactions, scarlet fever and pernicious anemia. Charcot-Leyden crystals, which are found in bronchial secretions from asthmatics, are derived from nucleoprotein-disintegration products of eosinophils.

Polymorphonuclear basophilic leukocytes (basophils) possess large cytoplasmic granules which stain a deep blue. These cells, which are primarily sources of blood heparin and histamine, constitute less than 1.0% of the leukocytes. Basophilic leukocytosis is seen in chronic myelocytic leukemia, hemolytic anemia and Hodgkin's disease. Basophilic leukopenia occurs following radiation or therapy with glucocorticoids.

Lymphocytes have a cell diameter from 7 to 10 μm (small) to 10 to 18 μm (large). They have a round, or slightly indented, deeply stained nucleus and normally comprise 25 to 33% of the leukocytes. Lymphocytosis is seen in infectious mononucleosis, lymphocytic leukemia, rickets and in most conditions associated with neutrophilic leukopenia (neutropenia).

Monocytes constitute 3 to 7% of the leukocytes. They are larger (12 to 20 μm) than the other leukocytes and possess an abundant, pale, bluish-violet-stained cytoplasm with a fine, reticulated chromatin structure in the nucleus. The monocytes (macrophages) phagocytize bacteria, parasitic protozoa, foreign particles and even erythrocytes. Monocytosis is seen in certain microbial infections (tuberculosis, typhus, malaria), Hodgkin's disease and monocytic leukemia.

Drug therapy frequently causes neutrophil dysfunction which can be characterized by a decreased number of mature neutrophils or a defect in cellular function resulting in the inability of the body to defend itself against infection. Drugs such as nitrogen mustard and chloramphenicol degenerate bone-marrow stem cells, and DNA synthesis is impaired by antimetabolites such as methotrexate and fluorouracil. Depolymerization of DNA is caused by procarbazine and alkylating agents. Mitosis is inhibited by colchicine and vinca alkaloids. The following outline lists drugs which cause granulocytopenia.[2]

- Nonchemotherapeutic
  - rifampin
  - fistocetin
  - benzene
  - nitrous oxide
  - ethanol
- Antithyroid
  - carbimazole
  - methimazole
  - thiouracil
- Diuretics
  - acetazolamide
  - chlorthalidone
  - chlorothiazide
  - ethacrynic acid
  - hydrochlorothiazide
  - mercurials
- Antihistamines
  - ethylenediamine
  - thenalidine
  - metaphenylene
  - pyribenzamine
- Phenothiazines
  - chlorpromazine
  - mepazine
  - methotrimeprazine
  - prochlorperazine
  - thoridizine
- Antibiotics
  - chloramphenicol
  - carbenicillin
  - griseofulvin
  - isoniazid
  - novobiocin
- Cardiovascular
  - diazoxide
  - procainamide
  - methyl dopa
  - quinidine
  - propranolol

As qualitative and quantitative changes in leukocytes in peripheral blood and their precursors in bone marrow and lymphatic tissue are associated with the various types of *leukemia*, this disease has been classified on the basis of the predominating type of leukocyte, ie, myelocytic (granulocytic), lymphocytic, monocytic or plasmacytic. Leukemia may be either acute or chronic and involve the replacement of bone-marrow elements by malignant cells, infiltration of the reticuloendothelial system, anemia, thrombocytopenia and hemorrhage. Leukemia usually is associated with an elevated WBC count and increase in the specific cell and its precursors in peripheral blood, but in certain instances there is an aleukemic blood picture with no evidence of leukocytosis. Leukocytes in acute leukemia are more immature ("blast"-type cells) than those encountered in the chronic type.

In many diseases of the hematopoietic system, it is necessary to examine the bone marrow to determine the rates of formation, maturation and release of blood cells into the peripheral circulation. Using a puncture biopsy needle, samples of *bone marrow* may be obtained from the sternum, iliac crest or proximal end of the tibia. Smears of marrow then are prepared, stained (Wright's stain or specialized histopathological procedure) and examined microscopically. The ratio of myeloid leukocyte to nucleated red cells in bone marrow, the presence of abnormal (*nonmyeloid*) cells, the number of platelet precursors (*megakaryocytes*), the signs of cell-maturation arrest and the presence of focal lesions are important factors in the diagnosis of various disease states.

Systemic lupus erythematosus (SLE) is a disease characterized by numerous clinical and pathological manifestations associated with various organs. Although the disease chiefly affects the lymphatic system, the cardiac, renal and articular systems also are involved. The diagnosis of this disease is based on the presence of an SLE-cell factor in the gamma-globulin fraction of blood in the diseased state. This factor dissolves the nuclei of leukocytes by depolymerization of deoxyribonucleic acid to form the SLE-body. If serum from patients with SLE is incubated with white cells, the "polys" will engulf the liberated SLE-body and form the typical SLE-cell with a characteristic progressive loss of nuclear detail. Drugs which cause SLE and produce a positive SLE-prep include hydralazine, procainamide, isoniazid and phenytoin.

These antibodies to nucleoprotein also can be detected by immunological techniques. In the double-antibody technique, the test serum containing antibodies to nuclear protein is incubated with a rat kidney slice (antigen). The second antibody is a fluorescein-labeled goat antihuman immunoglobulin (IgG) which combines with the human IgG bound to the antigen site in a positive test. The fluorescence is estimated by immunomicroscopy. Normal light-microscopy can be used if the goat-antihuman IgG is labeled with peroxidase.

Thrombocytes—The primary functions of *thrombocytes* (blood platelets) are the maintenance of hemostasis (arrest of blood flow from a vessel) and blood coagulation (clot formation). Platelets are oval to spherical in shape and have a mean diameter of 2 to 4 μm. They originate from an immature cell (megakaryocyte) in bone marrow and ranges of 140,000 to 450,000/cu mm have been reported in normal blood.

Adhesiveness, aggregation and agglutination are the principal physical properties of platelets responsible for hemostasis and coagulation reactions. Chemically, they contain protein (60%), lipid (15%) and carbohydrate (8.5%). Their content of serotonin, epinephrine and norepinephrine aids in promoting constriction at the site of injury. The release of "platelet thromboplastin," a cephalin-type phosphatide, and ADP are important in blood coagulation.

As of the present time, there is no satisfactory manual method for accurate enumeration of blood platelets. The size and physical properties of the platelet seriously deter the development of accurate and reproducible methodology. Indirect methods of analysis are based on the proportion of platelets to erythrocytes in a stained blood smear. Blood samples obtained directly from the fingertip puncture are diluted with an anticoagulant fluid which simultaneously will stain the platelets. The ratio of platelets to red cells then is determined microscopically and the number calculated from the predetermined red-cell count (normal 3 to 8 platelets/100 RBC). In the direct procedures, a sample of blood is obtained by venipuncture, placed in a siliconized

tube, diluted and subsequently analyzed by counting the platelets in a microscopic counting chamber using conventional or phase-microscopy apparatus. Suitable diluting fluids are the Rees-Ecker Fluid (sodium citrate, 3.8 g; formaldehyde, 0.22 mL; brilliant cresyl blue, 0.05 g; water, qs 100 mL) or Brecker Fluid (1% ammonium oxalate). Automated procedures for platelet counting have increased the accuracy to ±5 to 10%. Blood is collected in a special anticoagulant, diluted and centrifuged at specified speeds to obtain a "platelet-rich" supernatant fluid, which then is counted in an automated counting apparatus similar to those used for RBC counting.

Methods for counting platelets in whole blood include electronic impedence instruments and laser-optical counters using hydrodynamic focusing.[3] These new hematology multiparameter analyzers provide greater accuracy, precision and increased rate of analysis performed on a small volume of blood. The automated instruments provide precise platelet measurements for monitoring chemotherapy-induced thrombocytopenia and transfusion therapy.

Persistent increases in platelet count (*thrombocythemia* or *piastrenemia*) have been observed in chronic myelocytic leukemia, polycythemia, megakaryocytic hyperplasia and splenic atrophy. Acute or temporary increases in platelet values (*thrombocytosis*) are seen in trauma and asphyxiation.

Thrombocytopenia or a decrease in platelets to values less than 60,000/cu mm occurs in various purpuras or hemorrhagic states (idiopathic or symptomatic thrombocytopenic purpura). Inherited platelet defects include Glanzmann's thrombasthenia which is characterized by prolonged bleeding time and poor clot retraction, while Bernard-Soulier Syndrome and Von Willebrand's disease demonstrates defective platelet adhesiveness. Defects in the release reaction includes "Storage Pool Deficiency" and "Aspirin-like" syndrome.

A rare, inherited, structural and functional platelet abnormality is the *grey-platelet syndrome* characterized by large platelets lacking alpha granules and appearing grey on Wright's-stained peripheral blood smears. Patients have a history of bleeding, petechiae, easy bruising and expistaxis. Diagnosis is confirmed by radioimmunoassay procedures to detect levels of platelet-specific alpha-granule proteins.

Leukemia, extensive burns, splenic disorders and agents such as quinidine, sulfonamides, hydrochlorothiazide, diuretics, antiepileptics and neuropharmacological agents have been implicated in the etiology of symptomatic thrombocytopenia. Decreases in platelet count also are accompanied by morphological changes in the size, shape and cytoplasmic granulation of these cells and changes in adhesiveness and normal function in hemostasis and coagulation.

Studies on *platelet aggregation* have been of significant value in the study of platelet abnormalities and their role in disease states. The rate and extent of the aggregation and clotting response to adrenaline, ADP, collagen and thrombin have been measured by observing changes in optical density of platelet-rich plasma on adding of these agents or other test substances. Low amounts of ADP give reversible aggregation, while a biphasic-aggregation pattern occurs with intermediate concentrations of ADP or with epinephrine. The second phase is the release of the platelets' endogenous ADP. High concentrations of ADP result in an irreversible aggregation. Aspirin acts as an inhibitor of the intrinsic-platelet ADP and the collagen reaction.

Reticulocytes—In normal peripheral blood 0.5 to 1.5% of the erythrocytes possess a fine reticulum in the cytoplasm. In blood smears prepared with Wright's, Giemsa and other Romanowsky methods, basophilic stippling of the erythrocytes occurs in lead poisoning (*plumbism*). This is not to be confused with the basophilic staining of the reticulocyte which only can be seen when cells are stained by supravital procedures (mixture of dyes with wet blood prior to preparing of an air-dried blood smear). The observed granular filaments or reticulum of this immature erythrocyte are a result of endoplasmic coagulation by lipophilic dyes used in the supravital procedures. *Reticulocytes* are enumerated by supravital staining of fresh blood with an anticoagulant-dye solution.

The usual method of expression is

$$\% \text{ Retics} = \frac{\text{No of reticulocytes/1000 RBC}}{10} \quad (1)$$

The "corrected" reticulocyte count is calculated for a more meaningful clinical approach in the degree of anemia by expressing the percentage of reticulocytes per mm^3 of whole blood.

$$\text{Corrected reticulocyte count} = \text{Reticulocyte count} \times \frac{\text{(Patient's hematocrit)}}{\text{(Normal hematocrit)}}$$

In indirect counting methods a thin film of the blood-dye mixture is prepared on a microscope slide, counterstained with Wright's stain and the reticulocytes enumerated in proportion to a predetermined erythrocyte count. In direct procedures, reticulocytes are enumerated in wet films without counterstaining. Suitable dyes are brilliant cresyl blue, methylene blue and Janus green. These methods are subject to a high counting error.

An increase in the number of reticulocytes is an index of accelerated hematopoiesis and is observed in acute hemorrhage or adequate therapeutic management of iron-deficiency or pernicious anemia. In cases of chronic blood loss or bone-marrow depression a decrease in reticulocytes is seen.

Blood-Volume and Erythropoietic Mechanisms—The mean red-cell mass in normal males is 2095 ± 384 mL (30 mL/kg), the average plasma volume is 2766 ± 459 mL (40 mL/kg) and the total blood volume is 4861 ± 795 mL (70 mL/kg). The specific determination of *red-cell mass* is estimated accurately by tagging erythrocytes with ^{51}Cr *in vitro* or ^{59}Fe *in vivo*. These isotopes are incorporated into the β-polypeptide (Cr) or porphyrin (Fe) of hemoglobin in the RBC and subsequent isotope dilution in blood after injection of tagged erythrocytes is used for calculation of red-cell mass. In hemolytic anemia there is also a decrease in the normal life span (108 to 120 days) of the erythrocyte as indicated by a decreased survival time of ^{51}Cr-tagged red cells in blood (refer to Chapter 33).

Plasma volume is estimated by measurement of hemodilution of IV-injected ^{125}I or ^{131}I human serum albumin. The activity of labeled albumin steadily decreases after injection due to the loss of albumin to the extravascular space. Estimates of zero-time radioactivity levels can be made by extrapolation of a typical first-order blood-level decay curve. Dyes (Evans Blue) and other isotopes are less satisfactory for accurate assessment of plasma volume. The total blood volume is equal to the red-cell mass and plasma volume.

Chronic expansion of the red-cell mass is seen in primary and secondary polycythemia associated with erythrocytosis due to hypoxia, tumors and renal disease. In these conditions, there is an increased hemoglobin and hematocrit and absolute increase in red-cell mass. In relative polycythemia the high hematocrit is due to contraction of the plasma volume. *Chronic expansion of the blood volume*, with a resultant decrease in hematocrit value and, in some cases, a "hemodilution" anemia, is seen in cardiac failure, normal pregnancy, hepatic cirrhosis, splenomegaly and arteriovenous fistula.

The metabolic defect in *pernicious anemia*, characterized by inadequate gastrointestinal absorption of vitamin B_{12}, is diagnosed readily by monitoring urinary radioactivity fol-

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lowing oral administration of cyanocobalamin-^{57}Co with and without intrinsic factor. The percent recovery of the isotope in normal patients is 3 to 25% and in pernicious anemia 0 to 2.5%.

^{51}Cr-tagged erythrocytes also are used in studying the effects of various compounds, such as the nonsteroidal anti-inflammatory drugs, on *gastrointestinal (GI) bleeding*. The patient's blood cells are tagged with ^{51}Cr and the agent under test is administered. If GI bleeding occurs, there is an increase in ^{51}Cr content of fecal samples as a result of blood loss into the lumen of the GI tract.

Measurement of the absorption of radioactive iron (^{59}Fe), its tissue distribution (liver, spleen, precordium, sacral bone marrow), plasma elimination and urinary excretion establish various *ferrokinetic parameters*. Iron is absorbed to the greatest extent as the ferrous salt in the upper small intestine. Absorption is decreased in iron overload, erythropoiesis and various malignant, inflammatory or infectious diseases. Iron is transported in plasma bound to transferrin, a specific iron-binding protein. Alterations in plasma iron and iron-binding capacity are seen in pregnancy, thalassemia major and iron deficiency (hypochromic) anemia. Iron is stored in the liver, bone marrow, skeletal muscle and spleen as ferritin and hemosiderin. The daily turnover of iron is about 35 mg, primarily from an "erythropoietic labile pool" in bone marrow.

Hemosiderosis is simply an increase in iron storage, whereas *hemochromatosis* denotes increased iron storage with associated tissue damage. Both of these states can result from oral or parenteral medicinal/transfusion iron overload. Iron excretion is limited and occurs by desquamation of iron-containing cells from the bowel, skin and urinary tract.

Iron-deficiency anemia is a symptom and not a disease. Treatment is based on evaluation of ferrokinetic parameters, correction of hemoglobin and tissue-iron deficiency and recognition of the underlying cause (eg, chronic blood loss).

Blood Coagulation—*Hemostasis*, the arrest of blood flow from a vessel, is regulated by extravascular (muscle, skin and subcutaneous tissue), vascular (blood vessels) and intravascular (platelet-adhesion, clot-retraction and blood-coagulation) mechanisms. The following discussion will be limited to those processes related to the blood-coagulation mechanism. When blood is allowed to clot, the free-flowing liquid is converted into a firm cell clot surrounded by serum. If an anticoagulant is added to blood, coagulation does not occur and the blood cells are suspended in a liquid phase —plasma. The clotting mechanism involves three stages: the formation of plasma *thromboplastin*, the conversion of *prothrombin* to *thrombin* and the conversion of *fibrinogen* to *fibrin*.

The International Committee on Nomenclature of Blood Clotting Factors has numerically designated the blood-coagulation factors (Table II). Fibrinogen and Factors V and VIII are absent in normal blood serum as a result of the clotting process. The absorption characteristics of certain blood-coagulation factors on calcium phosphate or barium sulfate are used in the differential analysis of specific factors. The interaction of coagulation factors may be initiated through either the intrinsic or extrinsic pathways. In the intrinsic system all the factors are present in the blood, while the extrinsic system is activated by the release of tissue thromboplastin. Figure 28-3 shows the activities of both pathways to form a stabilized fibrin clot.

In Stage 1 of the coagulation process, the contact of injured tissue with blood results in the activation of Factor XII, which reacts with calcium, PTA, PTC, AHG and Factors III, V and X to yield intrinsic or blood thromboplastin. This stage normally is completed in 3 to 5 min. Extrinsic or tissue thromboplastin is formed rapidly (<12 sec) in various tissues in the body such as lung and brain in the presence of calcium and Factors V, VII and X.

Table II—Blood-Coagulation Factors

Factor	Synonym
I	Fibrinogen
II	Prothrombin
III	Thromboplastin (tissue)
IV	Calcium
V	Labile factor, proaccelerin, Ac globulin
VI	Accelerin
VII	Stable factor, proconvertin, serum prothrombin conversion accelerator (SPCA)
VIII	Antihemophilic globulin (AHG)
IX	Christmas factor, plasma thromboplastin component (PTC)
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent (PTA)
XII	Hageman factor
XIII	Fibrin-stabilizing factor (FSF)

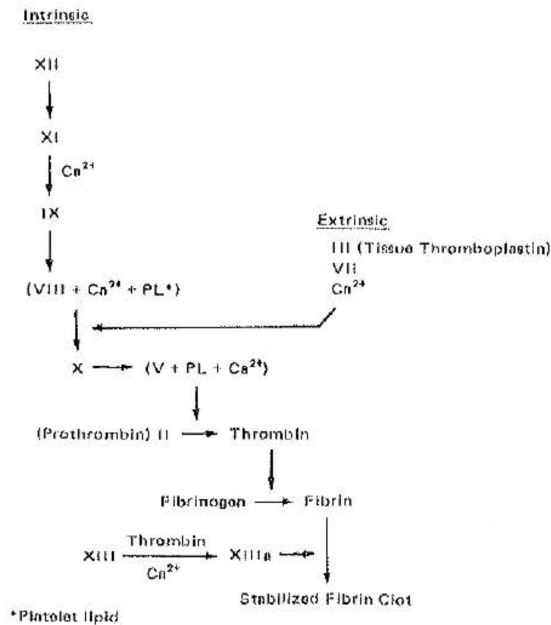

Fig 28-3. Blood Coagulation Process.

In Stage 2, thromboplastin catalyzes the conversion of prothrombin to thrombin (8 to 15 sec) in the presence of Factors V, VII, X and calcium.

In Stage 3, the thrombin rapidly converts fibrinogen into fibrin, which then forms a network of fibers that traps red cells and thus forms the blood clot.

Although the exact nature of the enzymatic sequences in the coagulation process is not clear, it is definitely a biological amplification process starting from the small reaction of tissue contact to rapid conversion of fibrinogen to fibrin.

Blood contains natural inhibitors of coagulation such as antithrombin, heparin and antithromboplastin, which can prevent a particular reaction in the coagulation sequence. The dissolution of blood clots occurs by the action of the blood proteolytic enzyme—plasmin or fibrinolysin. Plasmin is formed from its precursor, plasminogen, after activation by tissue and body fluids or substances of bacterial origin (streptokinase).

The routine tests performed in the coagulation laboratory are indices of vascular function (vascular phase and platelet adhesion) or intrinsic clotting mechanisms. Determinations of *bleeding time* and *capillary fragility* provide esti-

mates of blood coagulation in the presence of platelets and tissue or vascular factors. In the Ivy method for determination of *capillary bleeding time*, a blood pressure cuff is placed on the forearm and inflated to 40 torr; a puncture wound is made and the time required for bleeding to stop is noted. *Bleeding time* is a screening test for disorders of platelet function or vascular defects but is usually normal in coagulation disorders. The test is useful in the differential diagnosis of Von Willebrand's (reduced factor VIII, with a normal bleeding time) disease from mild hemophilia. The normal bleeding time, as determined by this method is 1 to 9 min. Dextran, pantothenyl alcohol, and derivatives, penicillin G, nonsteroidal anti-inflammatory drugs and streptokinase-streptodornase may cause a prolonged bleeding time. The *Simplate II* (General Diagnostics Div, *Warner Lambert*) is a standardized, disposable, springloaded bleeding-time device for platelet function testing. It uses two blades that are released automatically to produce two uniform incisions 6 mm long × 1 mm deep, making the procedure reliable and reproducible.

The *capillary fragility* or *tourniquet test* is based on the incidence of petechiae (small red marks) formation produced by an inflated blood pressure cuff over a 5-min period. Normally, a few tiny petechiae may appear. The most common cause of abnormalities in vascular-function and platelet-adhesion tests is thrombocytopenia.

An analysis of the *intrinsic coagulation mechanism* is concerned with the determination of the levels of the specific clotting factors in whole blood. In preliminary studies of a suspected hemorrhagic disorder, determinations of *coagulation time, clot retraction, platelet count, bleeding time* and *capillary fragility* usually are performed.

In the Lee-White procedure, the coagulation time of whole blood is determined in regular or siliconed tubes. Normal values are 8.5 to 15 min in glass and 19 to 60 min in siliconed tubes. Anticoagulants and tetracyclines may cause increased times while corticosteroids and epinephrine cause decreased values. The siliconization of glassware prevents platelet aggregation and thus, delays coagulation. The samples used in the analysis of coagulation time are then inspected at 0.5, 1, 2, 4 and 24 hr after clotting to determine the time required for the various phases of clot retraction. The tubes also are observed for evidence of clot lysis or dissolution. The clot normally will start to retract in 30 min, completely retract within 24 hr and show no evidence of lysis over a 72-hr period. Prolonged coagulation times are associated with hemophilia, hypofibrinogenemia and Factor IX deficiency. Abnormalities in any of these tests indicate the requirements for further coagulation studies.

The *prothrombin time test* is a measure of the levels of all coagulation factors, except III, IV and VII, and is an index of the capacity of plasma to form thrombin. In the "One Stage" test, the plasma sample is mixed with calcium chloride and tissue thromboplastin, and the time required for fibrin-clot formation is determined. Results are compared with a normal plasma control, and the prothrombin time is reported either in seconds or as the percent of prothrombin calculated from a standard activity curve. Correction studies using normal serum, adsorbed normal plasma or whole normal plasma added to test serum indicate deficiencies of Factors VII and X, Factor V and Factor II, respectively. If none of these additives shorten the prothrombin time, a circulating anticoagulant problem can be suspected.

A modification of this technique (the *prothrombin-proconvertin procedure*) using a 1:10 dilution of both patient and control plasma in the presence of prothrombin-free plasma as a source of Factors I and V, is a more sensitive index of specific deficiencies in prothrombin, Factor VII, IX and X.

Owren's *thrombotest*, as performed on whole blood, is sensitive to changes in both extravascular and intravascular clotting mechanisms, including Factor IX. The dosage of anticoagulant drugs, such as dicumarol, is adjusted in accordance with prothrombin-time determinations; patients are maintained usually within a therapeutic range of 20 to 40% of prothrombin activity (normal range, 80 to 130%). Reduced prothrombin levels, with prolonged prothrombin times, are observed in vitamin K deficiency, hemorrhagic disease of the newborn, excessive anticoagulant therapy, liver and biliary disease. The interaction of other drugs with anticoagulants may cause increased prothrombin times. Drugs such as salicylates, phenylbutazone, oxyphenbutazone, indomethacin and some sulfonamides increase the amount of active anticoagulant activity. Other drugs decrease the amount of vitamin K produced by gut bacteria which include chloramphenicol, kanamycin, neomycin, streptomycin and the sulfonamides.

The *prothrombin consumption test* is an index of the efficiency of conversion of prothrombin to thrombin in the coagulation process. The blood sample is allowed to clot under standardized conditions and then the quantity of prothrombin complex removed in the serum is determined in the presence of extrinsic fibrinogen. At least 80% of the prothrombin is consumed normally. Reduced consumption (<80%) is observed in coagulation deficiencies (hemophilia) related to thromboplastin generation.

Other types of coagulation tests detect deficiencies in *thromboplastin generation mechanism*. The *thromboplastin generation time test* (TGT) provides a means of detecting specific deficiencies of Factors V, VIII, IX, X, XI or XII. In the initial phase of this procedure the clotting time of the patient's adsorbed plasma is determined in the presence of a standardized platelet factor reagent, calcium chloride, plasma substrate reagent (Factors I, II and V) and the patient's serum. If the clotting time is abnormal (>16 sec), further tests are performed with the patient's plasma or serum. The adsorption of the plasma sample on barium sulfate removes Factors II, VII, IX and X and facilitates differentiation of a Factor IX to X from V to VIII deficiency in the thromboplastin-generation mechanism. Thromboplastin generation is reduced in hemophilia and thrombocytopenia.

The *activated partial thromboplastin time test* (PTT) is based on the observation that hemophilic plasma has a normal clotting time in the presence of a complete thromboplastin (extrinsic-saline extract of brain tissue), as used in prothrombin determinations, but will give a markedly prolonged clotting time with an incomplete thromboplastin (cephalin). Cephalin is a thromboplastic, ether-soluble phospholipid factor with platelet-like activity. In this test the clotting time of the patient's plasma is determined in the presence of calcium chloride and activated cephalin. This test is used primarily to detect deficiencies in Stage I of the coagulation mechanism and is rather sensitive to changes in Factors VIII and IX, as seen in classical hemophilia and Factor IX deficiency (Hemophilia B or Christmas disease).

In Stage 3 of the coagulation process, the presence of adequate levels of fibrinogen and thrombin is critical. *Fibrinogen levels* are analyzed semiquantitatively by determining the clotting time of a diluted plasma sample in the presence of extrinsic thromboplastin. This test is basically independent of prothrombin levels. Fibrinogen concentrations of 125 mg% or greater are adequate; deficiencies (hypofibrinogenemia) have been observed in liver disease, carcinomatosis and in certain complications of pregnancy.

Increased levels of *fibrinogen degradation products* (FDP) have been demonstrated in serum due to primary activation of the fibrinolytic system (pathological fibrinolysis) or by secondary activation following increased blood clotting (disseminated intravascular coagulation). Fibrinogen (mol wt 3.4×10^5) is degraded sequentially to fragments

X, Y, D and E with mol wts of 2.7, 1.65, 0.85 and 0.55 × 10^5, respectively. Fragments X and Y are more potent anticoagulants than fragments D and E and are responsible for hemorrhagic states in defibrination. Complexes between fibrin monomer, fragment X and other FDP interfere with thromboplastin generation and platelet formation. FDP can be measured by immunological techniques involving latex agglutination of particles sensitized with specific antibodies to FDP or by a hemagglutination-inhibition test. The normal level of serum FDP is 4.9 ± 2.8 μg/mL. Increased levels are seen in acute myocardial infarction, menstruation, complications of pregnancy, hypoxic newborns, malignancy and renal disease.

Deficiencies in the clotting mechanisms usually can be corrected partially and temporarily by transfusion of normal blood or plasma. When this fails, the presence of *circulating anticoagulants* (antithrombin, antithromboplastins, heparin) must be considered. Heparin acts indirectly by means of antithrombin III, which neutralizes several activated clotting factors (XIIa, activated Fletcher factor, XIa, IXa, Xa, IIa and XIIIa). The pharmacological effect of an oral anticoagulant is the inhibition of blood clotting by interfering with vitamin K-dependent clotting factors II, VII, IX and X. Circulating anticoagulants are detected by determining the effect of normal plasma on the clotting time (*recalcification time*) of the patient's oxalated plasma in the presence of calcium chloride. If the addition of the normal plasma does not shorten the prolonged recalcification time, a circulating anticoagulant state can be reported.

Since the end-point of all coagulation tests is the conversion of fibrinogen to fibrin, it is vital that the analyst rigidly standardize his concepts of fibrin formation in visual recording procedures. The use of mechanical instrumentation in the detection of clot formation significantly has increased the standardization, accuracy and reproducibility of coagulation procedures. These instruments measure and record the process of fibrin formation via increased turbidity (coagulogram or photometric clot detection) or changes in electrical conductance in the reaction mixtures. As well as performing routine laboratory tests simultaneously or sequentially, updated systems can run Fibrinogen and Factor assays achieving rapid throughput and accuracy. New performance features are available with many of the automated coagulation instruments. These include monitoring temperature zones, digital displays of the individual clotting times, automatic dilutions or patients samples and programmable parameters for testing flexibility.

Hemophilia is a classic deficiency of antihemophilic globulin (AHG), Christmas disease of PTC and Hageman trait of Factor XII. Hereditary or acquired deficiencies of Factors II, V, VII, X and XI also are associated with disease states. The process of blood coagulation, analysis of coagulation factors and interpretation of results comprise a highly complex system. The coagulation laboratory and the physician function together in the diagnosis and treatment of coagulation-deficiency diseases.

Blood-Bank Technology

Blood-bank technology in the modern laboratory is part of the blood-transfusion service. As whole blood for transfusion and its components are biologically active therapeutic substances, a complete analysis of their chemical and biological characteristics is vital to the assurance of successful therapeutic effects. The transfusion service is responsible for:

1. Receiving and examining of the donor.
2. Collecting, processing and storing the blood.
3. Typing of recipient and donor for ABO and Rh blood-group factors.
4. Compatibility (cross-matching) testing before transfusion.
5. Issuing of blood for transfusion and extracorporeal circulation.
6. Evaluating transfusion complications.
7. Performing of special serological tests pertinent to blood groups and other factors.

In this section a discussion of pertinent factors related to the various phases of the transfusion service will be presented.

Receiving and Examining of the Donor—A complete registry[4] of prospective donors should be maintained, with specific reference to age, sex, weight, address, occupation and telephone number. Computerized blood banking has increased the efficiency of this service. Donors should preferably be between the ages of 21 and 60 and should weigh no less than 110 lb. The donor may be rejected on the basis of previous or active incidence of certain microbial diseases (recurrent malaria, syphilis, infectious or homologous serum hepatitis, tuberculosis), bleeding abnormalities, convulsions, allergic syndromes, skin or heart diseases, diabetes, alcohol or drug addiction, pregnancy, cancer, recent immunization with live vaccine product, acquired immune deficiency syndrome (AIDS) or blood-pressure abnormalities (acceptable blood pressure: between 100/50 and 200/100; pulse rate: 60 to 12/min). The screening of blood for exposure to human immunodeficiency virus (HIV) is crucial to reducing the risk of infection from transfusion. ELISA (enzyme-linked immunosorbent assay) screening tests for the detection of antibodies against HIV are available from manufacturers. More sensitive tests are being developed to detect viral DNA in body fluids.

A period of at least 8 weeks should have elapsed since blood was withdrawn and the blood hemoglobin level should be 12.5 to 13.5 g% or greater. Serum bilirubin and transaminase levels also should be evaluated in donors with previous incidence of jaundice.

Collecting, Processing and Storing the Blood—A tourniquet is applied to the arm of the donor to occlude the venous return, the skin area is sterilized and the blood is collected by venipuncture (phlebotomy). NIH Formula A or B [ACD(Acid-Citrate-Dextrose) or ACD-phosphate] solutions are used as anticoagulants in the sterile blood-collecting containers. Evacuated containers may be of regular or siliconed glass; collapsible plastic containers offer many advantages in donation, blood-banking and transfusion procedures.

The preservation of the red cells in blood is improved by the complete removal of trapped air in the blood-collection apparatus, rapid cooling after collection and storage at 4°. Properly collected whole blood is usually stable for 21 days at 1 to 6°. The deterioration of whole blood is related to increased cellular fragility (increased plasma K^+) and decreased glucose utilization. Blood which is used for correction of any bleeding tendency or clotting defect should be as fresh as possible. Leukocytes, platelets and Factors V and VIII deteriorate in stored plasma or whole blood.

ABO Blood-Group Classification[5]—Human red cells can be classified into various groups or types on the basis of reactivity of certain blood factors (*agglutinogens*) located on the erythrocyte membrane. The Landsteiner system (Table III) for the four blood groups is based on the presence or absence of either A or B agglutinogen on the cell surface (Group A, B, AB or O, respectively).

Serum does not contain the antibody (*agglutinin*-IgM type) for the antigen present in an individual's own red cells, but does contain the isoagglutinin (eg, anti-B in blood group A) due to exposure, early in life, to bacterial and plant antigens similar in structure to the A-B antigens. The clumping or agglutination of the red cells by reaction of agglutinogen with agglutinin is used in blood-grouping techniques. In certain instances hemolysin antibodies, present

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Table III—Blood-Group Systems

Blood Group	Agglutinogen in Cell	Agglutinin in Serum	Reaction[a] with Anti-A Serum	Reaction[a] with Anti-B Serum	Frequency (%) in Causasians
A	A	Anti-B	+	−	41
B	B	Anti-A-A_1	−	+	10
AB	AB	None	+	+	4
O	None	Anti-A and B	−	−	45

[a] Agglutination.

in serum containing anti-A or anti-B agglutinins, cause the disruption of cells and release of hemoglobin (hemolysis).

Human blood cells are grouped by two separate reactions: cellular or "front" grouping and serum or "reverse" grouping. The blood group ordinarily is determined by testing an individual's red cells with standardized anti-A or anti-B serum (certified by the Div of Biological Standards, NIH). Confirmation of the blood group (reverse typing) is accomplished by an analysis of an individual's agglutinin titer. In this procedure the individual's serum is heated at 56° for 10 min to destroy hemolysins, and then mixed with known Subgroup A_1 or B_1 human red (Rh-negative) cells in the agglutination test. These two tests should be in agreement prior to the release of blood for transfusion.

Although human blood cells of Group B react uniformly with Anti-B serum, Group A and AB cells show a wide range of reactivity with Anti-A or Anti-A_1B serum. Blood-group A may be further categorized into Subgroups A_1, $A_{int.}$, A_2, A_3, A_0 and A_x on the basis of the reaction with absorbed Anti-A, Anti-A_1-lectin, Anti-H-lectin, Anti-$A_{1,2}$ and Anti-AB serum and the presence of Anti-A_1 in the serum. Certain Group O individuals possess anti-H in their serum and are further subcategorized into the Bombay or O_h phenotype. Tests for A, B and H in saliva can establish the genotype of an individual, ie, A and H in saliva of blood-group A, B and H in B, H and O and A, B, H in AB. This is helpful in cases of poorly developed red-cell antigens or in the loss of cellular antigen in some patients with leukemia.

As the human blood cell contains many antigens with rather complex biochemical and immunochemical properties, the blood factors have been classified further into various subsystems. The Kell (K), Lutheran (Lu), Lewis (Le), Duffy (Fy), Kidd (Jk), MNS, Sutter (Js), Diego (Di) and P blood-factor systems are based on the detection of a specific antigen on or within the red cell by means of antibody (*isohemagglutinin*) reactions with specific antisera or panels of reagent red cells. Some of these factors (eg, Kidd, Kell and Lewis) have been involved in transfusion reactions.

The Rh-Hr System and Antihuman Globulin Test—The presence or absence of *Rh_0 antigen* in human blood is of prime importance in transfusion reactions, paternity disputes and isosensitization phenomena. There are eight blood Rh phenotypes which are determined by their reaction with three specific serum agglutinins (Anti-Rh_0, Anti-rh′ and Anti-rh″): rh, rh′, rh″, rh′rh″, Rh_0, Rh_0′, Rh_0″ and Rh_0′Rh_0″. The rh groups do not contain the Rh_0 factor on the cell surface and are designated "Rh-negative." The terminology of the Wiener system (Rh, rh) is comparable to the Fisher-Race (CDE) as follows: rh′(C), Rh_0(D), rh″(E). The Rosenfeld system uses a numerical classification: RH1 = Rh_0.

The absence of the Rh antigen in about 15% of the population does not preclude the presence of other factors; the use of specific antisera (Anti-hr′ and Anti-hr″) has demonstrated the existence of the Hr factors (Hr_0, hr′, hr″). For example, the Rh-negative cell (rh″) possesses rh″hr′Hr_0 antigens. The antigen Rh_0(D) is the most potent immunogen of all the Rh antigens.

The Rh antibodies are either *saline agglutinins* (complete) or "*blocking*" *antibodies* (incomplete). The latter are of the IgG type. They are used in Rh testing procedures and are produced more commonly, and in higher titer, in the human isosensitization or autoantibody reactions. They will not agglutinate saline suspensions of normal Rh-positive red cells except in the presence of a high concentration of albumin, serum or conglutinin (AB serum with albumin) at a temperature of 35 to 37°.

In routine Rh testing procedures, a sample of blood (oxalated or heparinized) or a suspension of cells in serum or albumin is mixed with Anti-Rh_0 serum on a slide or in a tube at 37 to 47°. The presence of clumping indicates that the blood possesses Rh_0 antigen. Confirmation of an Rh-negative test may be performed by retesting with Anti-rh′Rh_0rh″ serum.

In Rh testing procedures, red cells from patients with acquired hemolytic anemia are partially coated with human autoantibody, and cells from erythroblastic infants are coated with maternal antibody globulins and may be clumped falsely by Rh typing serum containing a high protein concentration, or may appear to be Rh-positive in the saline-cell suspension test. Demonstration of anti-Rh_0(D) in an eluate from these antibody-coated cells can help to establish true Rh type.

Anti-Rh antibodies are not normally present in human serum; they may be acquired via isosensitization. The transfusion of Rh-positive blood to an Rh-negative recipient, or transfer of cells of Rh-positive fetus through the placental barrier to the Rh-negative mother, will result in formation of antibodies to Rh agglutinogens not present in the cells of the recipient or mother, respectively.

Hemolytic blood-transfusion reactions and hemolytic disease of the newborn (erythroblastosis fetalis) involve *isosensitization phenomena* usually related to the Rh_0 antigen. Hr and ABO antigens also can be responsible for hemolytic disease of the newborn. If an expectant mother is Rh-negative and the father is Rh-positive, the Rh genotype of the father should be determined. If the father is homozygous, the erythrocytes will contain a pair of Rh_0 factors and the offspring will inherit the Rh_0 factor; if he is heterozygous, one Rh_0 and one Hr_0 factor will be present and his offspring may or may not inherit the factor.

If the fetus is Rh-positive, the mother may be sensitized to the Rh antigen and in subsequent pregnancies the development of high titers of Anti-Rh_0 antibodies will result in hemolytic disease of the fetus. These antibodies enter the fetal circulation via the placental barrier, coat the red cells of the fetus and cause excessive erythrocyte destruction, hyperbilirubinemia and associated potential for brain damage, hydrops fetalis (edema) and congenital anemia of the newborn. This Rh disease can be avoided now by proper therapeutic use of Rh_0(D) Human Immune Globulin (RhoGAM, *Ortho*) to prevent the postpartum formation of active antibodies in the Rh_0(D)-negative, D^u-negative mother who has delivered an Rh_0(D)-positive or D^u-positive infant.

The *Coombs' antiglobulin test* is a method of detecting the blocking-type antibodies, globulins and complement which are attached to red-cell antigens in isosensitization phenomena.

In the "direct" test procedure, a saline suspension of washed red cells is mixed with anti-human gamma globulin antiserum and agglutination is indicative of the combination of human antibody with antigen on the red cell, eg, maternal incomplete isoantibody on infant's red cells in hemolytic disease of the newborn, autoimmune, drug-induced, alloantibody-induced hemolytic anemia and after transfusion of incompatible red cells.

An "indirect" procedure is used to demonstrate the presence of blocking antibody in the serum of pregnant Rh-

negative women and in transfusion reactions. In this procedure the patient's serum is incubated with a suspension of Group O Rh-positive red cells; the cells are washed and then antihuman globulin antiserum is added to detect the coating of the red cells with antibody globulin from the patient's serum by agglutination phenomena. If agglutination occurs in the first part of the procedure, a saline agglutinin is also present. Anticomplement sera (anti-nongammaglobulin antiserum) are used to detect reactions involving anti-JK.

The Du allele is a clinically important variant of the Rh_0 factor and usually associated with rh′(C) and rh″(E). Individuals with this factor are considered Rh-positive, and the red cells fail to react with anti-Rh_0 in the saline-tube method but reacts with incomplete anti-Rh_0(D) by other slide or tube techniques. Rh-negative donors should be tested for Du factor. If positive, their blood must only be given to Rh-positive recipients.

Drug-Related Problems—Hematological abnormalities may be caused by the administration of drugs which can cause a positive direct antiglobulin test and immune hemolytic anemia, eg, cephaloridine, cephalothin (*Keflin*), methyldopa (*Aldomet*), penicillin, L-dopa, quinidine, phenacetin and insulin.

Compatibility Testing—Cross-matching procedures are designed to detect incompatibilities in the blood of donors and recipient. The test is designed to prevent transfusion reaction and assure maximum benefit to the patient. Although erroneous ABO grouping usually will result in an incompatible cross match, no such protection exists in the Rh system. An incorrectly typed Rh-positive donor blood can result in primary immunization to Rh_0(D) antigen if transfused to an Rh-negative recipient. For each transfusion, a *major* and *minor cross match* should be performed.

In the *major cross match* (1) a saline suspension of the donor's cells is mixed with the recipient's serum and (2) the donor's cells are suspended in recipient's serum or in serum with added albumin. The saline cross match is an additional check on the ABO typing and may detect incompatibilities caused by antibodies to M, N, S, P and Lu subgroups. The high-protein or albumin cross match can demonstrate antibodies in the Rh system. The presence of agglutination or hemolysis indicates incompatibility.

The *minor* cross match includes the donor's serum and the recipient's cells, and is useful as a check of the ABO typing and an indication of the possibility of transfusion reactions caused by a rare antigen on the recipient's cells or uncommon antibodies directed against an antigen in the serum of the donor. The minor cross match has been replaced in many instances with screening of the donor's serum against a panel or pool of red cells of known antigenicity.

The *indirect antihuman globulin* procedure also must be performed with the recipient's serum and donor's cells with and without albumin (major side) and may be tested with the donor's serum and recipient's cells (minor side). The use of proteolytic enzymes (bromelain) enhances the agglutination of red cells by low-titer or weakly reacting Rh-Hr antibodies, probably by removing sialic acid residues on the RBC surface. The red cells used in the indirect Coombs test are treated with the enzyme prior to absorption of antibodies and addition of antiglobulin reagent.

The usual cross-matching techniques involve (1) a room-temperature or 30° procedure, preferably with the addition of albumin, (2) a high-protein procedure and (3) an antiglobulin procedure.

The presence of nonspecific *autoantibodies, cold agglutinins* and *bacteriogenic agglutination* sometimes complicates the cross-matching procedure. If the recipient's serum reacts more strongly with his own cells than with the donor's, autoantibodies should be suspected. Cold agglutinins usually will agglutinate all blood, regardless of type, at low temperatures, but will not react at 37°. Agglutination as a result of bacterial contamination of blood is called panagglutination.

Hepatitis Testing—Posttransfusion hepatitis is associated with the transmission of virus-like particles referred to as *Australia or serum hepatitis antigen or the hepatitis associated antigen* (*HAA*). All donor blood must be tested for the presence of HAA. Agar gel diffusion (AGD), counterelectrophoresis (CEP), complement fixation (CF) and rheophoresis procedures can be used.[6] The rheophoresis procedure uses a modified gel-diffusion technique for the detection of HAA by precipitin-type reaction with HAA antibody. It offers the sensitivity of CEP and CF procedures with the simplicity of the AGD procedure. Other tests for HAA are based on radioimmunoassay (RIA) technique for detection of antigen by hemagglutination (HA) or HA-inhibition for the presence of HAA antibody. In the RIA technique, the donor's serum is added to a test tube coated with HAA antibody (solid RIA). If the serum contains HAA, it will bind to the antibody. ^{125}I-HAA is then added to the tube. If the antibody binding site is occupied previously with HAA from the donor's serum, ^{125}I-HAA will not bind and the determination of ^{125}I bound versus free is an index of HAA content of the donor's serum.

Issuing of Blood and Evaluating Transfusion Reactions—Whole-blood, red-cell or leukocyte suspensions, plasma, platelet-rich plasma, platelet concentrates, leukocyte-poor blood, AHF, factor IX complex, plasma protein fractions and RhoGAM are products of the transfusion service.[7] Transfusion reactions are related to antibody phenomena or disease transmission. The hemolytic reaction resulting from the transfusion of incompatible cells is the most serious problem. The transfusion of microbially contaminated blood can result in a pyrogenic reaction or transmission of infectious diseases, such as malaria, syphilis or hepatitis. Allergic reactions (urticaria, asthmatic seizures), circulatory overload, embolic complications (blood clot, air emboli) also may be encountered. Leukocyte and platelet antibodies develop in repeat transfusions and in transplantation patients. The transfusion service is an integral unit in evaluating such complications.

Techniques of Analysis

This section will describe the principles of the procedures used in the analyses of various substances in blood, plasma or urine. Examples of the significance of such tests in clinical diagnosis will be presented. For a complete description of the physiological and pharmacological aspects of these blood constituents, see the *Bibliography*.

Instrumentation—The development of instrumentation has accelerated progress in clinical chemistry. An excellent review of the principles and applications in clinical chemistry of automation, atomic-absorption spectroscopy, ultraviolet and visible spectrophotometry, fluorimetry, phosphorimetry, infrared and Raman spectroscopy, microwave and radiowave spectroscopy and nucleonics was prepared by Broughton and Dawson.[8] Quality-control techniques are a vital part of any clinical laboratory. Standard reference materials,[9,10] standardization of quantities and units[11] and continual evaluation of precision and accuracy of various determinations[12] are incorporated into procedures of all reliable clinical laboratories. The manufacture of certified standards and reagents and the certification of clinical chemists and clinical laboratories are under the supervision of either the FDA, NIH, Pharmaceutical Manufacturers Association (PMA), American Association of Clinical Chemists, the College of American Pathologists and the National Committee for Clinical Laboratory Standards (NCCLS).

Interaction of Drugs with Clinical Laboratory Tests—Drugs may interfere with the interpretation of laboratory tests by three classes of mechanisms:

I. *Chemical or biochemical* interference due to reaction of a drug or its metabolite in biological fluids with test reagents in analytical procedures.
II. *Pharmacological* interference due to normal drug-induced alterations in various physiological parameters.
III. *Toxicological* interference as a consequence of the toxicity of a drug.

Examples of Class I interference include false-positive urine glucose results due to the reducing properties of drugs or metabolites such as ascorbic acid, *p*-aminosalicylic acid, tetracycline, cephaloridine and levodopa, which are excreted in urine. Spironolactone will result in an elevation of certain urinary ketosteroids through cross-reaction of the drug in the analytical procedure.

Examples of Class II interference include the decrease in serum-potassium levels in patients receiving thiazide diuretics, the alteration in serum uric acid with probenecid and the elevation in various plasma proteins and thyroid function tests with estrogen-progesterone combinations. Drug-drug interaction also can result in changes in these parameters. Guanethidine enhances the effect of the coumarin anticoagulants. Barbiturates induce hepatic microsomal enzyme synthesis and subsequently increase the metabolism and decrease the therapeutic effect of drugs, such as warfarin, even after these drugs are terminated.

Examples of Class III interference include changes in liver- and kidney-function tests and hematological parameters (anemia, agranulocytosis, leukopenia) due to drug-induced toxicity and positive LE and ANA tests due to a "lupus-like" syndrome induced by hydralazine.

It is beyond the scope of this chapter to include a complete listing of drug interactions in laboratory tests. The reader is referred to an annual, readily available, computerized review of the effect of normal therapeutic drug doses, as well as overdoses, on clinical laboratory tests[13] and to other review articles.[14]

Blood

Collection and Preparation for Chemical Analysis—Using aseptic technique, a blood sample is obtained by venipuncture and usually placed in evacuated glass tubes. The choice of anticoagulant, type of specimen, stability of test component and use of preservatives depends on the type of analysis requested and the specific analytical procedure involved. If serum is desired, the blood sample is allowed to clot and the serum is separated by centrifugation. When whole blood or plasma is to be used in the analysis, an anticoagulant is added to the collecting tube.

The following concentrations of specific anticoagulants are used routinely per 10 mL blood; lithium, potassium or sodium oxalate (15 to 25 mg), sodium citrate (40 to 60 mg), heparin sodium (2 mg), disodium or tripotassium ethylenediaminetetraacetate (EDTA-Na_2, 10 to 30 mg) or ACD-Formula B solution (1.0 mL).

Heparin prevents blood coagulation by inhibiting the thrombin-catalyzed conversion of fibrinogen to fibrin. The other anticoagulants either precipitate blood calcium or convert ionized calcium into a nonionized (chelated) form which cannot function in the coagulation reaction. Heparin and EDTA do not alter the cellular elements of blood significantly. Sodium fluoride and thymol are used as preservatives or enzyme inhibitors to prevent the deterioration of various substances in the blood sample, eg, glucose → lactic acid. Preservatives and anticoagulants can interfere with some enzyme tests. Serum usually is used for these procedures.

The separation of plasma or serum, and chemical analysis, usually are performed as soon as possible after the collection of the sample. The addition of polystyrene granules to the blood sample prior to centrifugation facilitates the isolation of serum or plasma. Hemolysis interferes with analytical procedures for bilirubin, albumin, nonprotein nitrogens, pH, phosphorus, potassium and various enzymes. The serum also should be observed for presence of lipemia. Changes in the ratio of CO_2, chloride and electrolytes in cells and plasma, glycolytic conversion of glucose to lactic acid, hydrolysis of ester phosphate to free inorganic phosphate, bacterial conversion of urea to ammonia and conversion of pyruvate to lactate are examples of changes that can occur in contaminated, improperly preserved or unrefrigerated blood specimens.

The first stage in many of the chemical determinations is the removal of blood protein and preparation of *protein-free blood filtrate*. The protein is precipitated with tungstic acid, trichloroacetic acid, zinc hydroxide or organic solvents, such as alcohol and acetone, and then filtered or centrifuged to remove the protein coagulum. Tungstic acid precipitation is performed by mixing 1 volume of blood or 2 volumes of plasma with 9 volumes of stabilized tungstic acid reagent. The filtrate obtained in this procedure should be in the pH range of 3.0 to 5.1 to assure the adequate removal of proteins (<2 mg% in filtrate).

The Somogyi filtrate is prepared by mixing 1 volume of blood with 5 volumes of water, 2 volumes of 5% zinc sulfate and 2 volumes of 0.3 *N* barium hydroxide. The barium sulfate is precipitated and the zinc hydroxide formed in the reaction precipitates the blood proteins. Trichloroacetic acid (10%), in a ratio of 9:1 with blood, yields greater volumes of filtrate due to a more complete formation of protein agglomerates.

Blood Glucose—Methods for determining blood glucose are based on the use of glucose as a reducing agent or on the enzymatic oxidation of glucose to gluconic acid. In the Folin-Wu technique, glucose is determined in a protein-free blood filtrate by reduction of alkaline cupric sulfate and subsequent reaction with phosphomolybdic or arsenomolybdic acid reagent to form a blue complex which can be estimated colorimetrically. The Nelson-Somogyi method uses a protein-free blood filtrate prepared with zinc hydroxide to remove most of the interfering reducing substances.

The presence of a terminal aldehyde in the glucose molecule is the basis of a colorimetric determination with phenolic hydroxyl reagents (phenol in aqueous methyl salicylate or phosphorylated 1,3-dihydroxybenzene) in the presence of strong sulfuric acid and heat.

The *o*-toluidine procedure is a color reaction specific for hexoses—glucose, mannose and galactose. Since aldohexoses other than glucose are normally present in very small concentrations, results obtained by this method approach the true value of glucose. *o*-Toluidine is condensed with glucose in glacial acetic acid to yield a green chromogen by forming an equilibrium mixture of a glycosylamine and Schiff base.

In the preceding techniques, interfering substances such as lactose, galactose and glutathione are measured and the value is reported in the nonspecific term "sugar." Enzymatic determination with glucose oxidase is the only test specific for blood glucose. Blood glucose is converted to gluconic acid and hydrogen peroxide by glucose oxidase; the peroxide is then estimated by iodimetric procedures or by oxidation of a chromogen (*o*-dianisidine or 2,2′-azino[diethylbenzothiazolinesulfonic acid]) in the presence of a peroxidase to form a colored product. Drugs which cause a slight increase in glucose values include ACTH, corticosteroids, D-thyroxine, diazoxide, epinephrine, estrogens, indomethacin, oral contraceptives, lithium carbonate, phenothiazones, phenytoin, thiabendazole and diuretics. Drug interferences with *o*-toluidine methods, which cause a slight increase, include

ascorbic acid, dextran, fructose, galactose, mannose, ribose, xylose and bilirubin.

Another enzymatic procedure uses the hexokinase-catalyzed conversion of glucose to glucose 6-phosphate (G6P), and then to 6-phosphogluconate and NADPH in the presence of NADP and G6P dehydrogenase. The NADPH thus formed is equivalent to the amount of glucose present and is estimated spectrometrically at 340 or 366 nm.

Normal fasting blood-sugar values for adults are 80 to 120 mg/100 mL; true glucose is 65 to 100 mg/100 mL. When the blood-sugar values exceeds 120 (hyperglycemia), diabetes mellitus should be suspected and can be confirmed by evidence of diminished carbohydrate tolerance. The effect of ingested carbohydrate on blood sugar can be determined by the *glucose tolerance test;* 100 g of glucose (1.75 g/kg) in water or a flavored beverage, is administered orally and glucose determinations are performed on blood and urine samples at hourly intervals for 3 hours. Values above 160 at 1 hr and 110 at 2 hours in blood samples are abnormal. The renal threshold for glucose is 180 to 200 mg/100 mL of blood, and, therefore, sugar should not appear in the urine of normal subjects in the tolerance test.

Hyperglycemia and decreased glucose tolerance are seen in diabetes mellitus (to 500 mg/100 mL) and hyperactivity of the adrenal, pituitary and thyroid glands. *Hypoglycemia*, with a blood-sugar value of <60 mg/100 mL and increased glucose tolerance, is encountered in insulin overdose, glucagon deficiencies and hypoactivity of various endocrine glands. Intravenous glucose tolerance studies are used to circumvent defective absorption of glucose in the gastrointestinal tract, eg, in steatorrhea.

Monitoring hemoglobin A_{1c} is another way to follow patients with hyperglycemia. This is more specific for diagnosing diabetes but less sensitive than the glucose tolerance test.[15] Normally, hemoglobin A_{1c} accounts for 3 to 6% of the total hemoglobin while in diabetics it is 6 to 12%. The concentration of Hgb A_{1c} in the blood reflects the patient's carbohydrate status over a period of time, providing a marker for hyperglycemia. *Pancreatic function tests* include studies on IV and oral glucose, glucagon and tolbutamide tolerance. The beta cells of pancreatic islet tissue secrete insulin and the alpha cells secrete glucagon, a substance antagonistic to insulin and having a hyperglycemic effect induced by its glycogenolytic action. In *glucagon tolerance studies* the effect of parenteral administration of glucagon on blood-sugar values is useful in the diagnosis of pancreatic and hepatic function. *Insulin and tolbutamide tolerance studies* are used in the diagnosis of endocrine disorders, differentiation of insulin-resistant diabetics and determination of functional hypoglycemia and islet-cell tumors.

Galactosemia, the presence of galactose (>4.5 mg%) in blood, is usually due to an inborn error of galactose metabolism. Congenital deficiencies in galactokinase or galactose 1-phosphate uridyl transferase result in inadequate galactose metabolism with accumulation of galactose 1-phosphate in the liver. Oral administration of galactose in galactosemia leads to a decrease in blood glucose and an increase in concentrations of galactose in the urine and blood. Galactose is measured by estimation of NADH liberated in the conversion of galactose to galactonolactone in the presence of NAD and galactose dehydrogenase. Deficiencies in intestinal disaccharidases such as lactase will preclude efficient conversion of lactose to galactose and glucose, and oral administration of lactose will cause no increase in blood galactose and usually produce diarrhea. Galactose-loading studies are useful in the diagnosis of toxic or inflammatory conditions of the liver. In hepatic cirrhosis, there is a decrease in the galactose-metabolizing capacity of the liver due to the inhibition of hepatic diphosphogalactose-4-epimerase.

Lactic acid is a product of glucose metabolism; it is converted into pyruvic acid and NADH by lactic dehydrogenase (LDH) in the presence of NAD. Blood lactic acid is estimated by reaction with LDH to form pyruvate and NADH; the NADH level is determined spectrophotometrically at 340 nm and is a function of lactic acid concentration. It is elevated (>20 mg/100 mL) following exercise, anesthesia and certain types of acidosis. The *blood lactate/pyruvate* ratio should be calculated in order to determine the presence of excess lactic acid in the blood in acidosis, thiamine deficiency and decompensated heart disease.

Blood pyruvic acid is determined by the reverse procedure; ie, the conversion of pyruvate to lactate in the presence of LDH and NADH. Normal blood pyruvic acid ranges from 0.6 to 1.3 mg/100 mL by chemical methods and 0.3 to 0.7 mg/100 mL by enzymic procedures.

Nonprotein Nitrogen (NPN) Compounds—These refer to all nitrogen-containing compounds in biological fluids exclusive of protein, including nitrogen from amino acids, low-molecular-weight peptides, urea, nucleotides, uric acid, creatinine, creatine and ammonia. Blood NPN usually is determined by digesting a protein-free blood filtrate with sulfuric acid in the presence of a catalyst (SeO_2) to convert nitrogen to ammonium sulfate (Kjeldahl digestion—see page 444); the excess acid is neutralized and ammonia determined by Nesslerization or reaction with alkaline hypochlorite.

The normal blood NPN is 25 to 45 mg/100 mL (48% urea N, 14% amino acid N, 4% creatine N, 1% creatinine N, 3% uric acid N and 30% residual N). In renal damage, NPN is elevated to values ranging from 60 to 500 mg/100 mL (*azotemia*). As variations in NPN mainly reflect alterations in blood urea nitrogen (BUN), urea determinations are more sensitive and preferred as a guide to kidney function.

The primary pathway of nitrogen metabolism in man is the synthesis of urea from ammonia in the liver and then rapid renal excretion of urea. In renal disease (*nephritis*), the excretion of urea is diminished and blood NPN and BUN are increased. In BUN procedures, *urea* is converted enzymatically to ammonia by urease; the ammonia then is determined by Nesslerization, reaction with phenol-alkaline hypochlorite, aeration into standard acid and subsequent titration or reaction with salicylate-nitroprusside reagent at pH 12 in the presence of alkaline dichloroisocyanurate to form a green chromogen which can be estimated colorimetrically. The ammonia also can be estimated by spectrophotometric determination of NAD produced in the conversion of ammonia and α-ketoglutarate to glutamate by NADH-L-glutamate dehydrogenase. Direct chemical determinations of urea are based on the reaction with 2,3-butanedione in an acid medium (Fearon reaction).

BUN (normal = 5 to 25 mg/100 mL) is increased in chronic and acute nephritis, metallic poisoning and cardiac failure; reduced levels occur in rapid dehydration or following diuresis. In severe liver damage due to diminished urea formation, an increase in blood ammonia and decrease in BUN are observed. Urine urea output (6 to 17 g/day) is an index of *glomerular filtration rate* (*GFR*) and kidney function. Increased dietary protein and gastrointestinal hemorrhage will increase urine urea. Decreases in urea excretion involve either tubular reabsorption or secretion defects.

The *nitrogen balance* represents the balance between nitrogen input or produced (N_{in}) and nitrogen excreted (N_{out}); in normal individuals $N_{in} = N_{out}$. N_{out} is regulated by renal GFR; in renal disease GFR is decreased, $N_{in} > N_{out}$ and BUN is increased. The rate of urinary excretion of parenterally administered dyes (phenolsulfonphthalein), inulin sodium, *p*-aminohippurate and mannitol are sensitive indices of GFR in *renal clearance studies*.

Creatine (methylguanidoacetic acid) and *creatinine* (creatine anhydride) are involved in the physiology of muscle

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contraction. Creatine phosphate is an intracellular source of high-energy phosphate bonds via the reaction of ATP and creatine kinase. Creatinine is the waste product of creatine metabolism and is the normally excreted compound.

Serum creatinine is determined by reaction with alkaline picrate to form a red chromogen. These values usually represent 20 to 30% of noncreatinine-interfering substances. Absolute determinations can be made by the absorption of creatinine from protein-free blood filtrates on aluminum silicate prior to the final determination. Drugs causing nephrotoxicity result in a slight increase in creatinine and those which interfere with color formation in the reaction include bromosulfophthalein (BSP), phenolsulfonphthalein (PSP), acetoacetate, ascorbic acid, levodopa, methyldopa, glucose and fructose. Creatine is determined after hydrolytic conversion to creatinine with boiling, aqueous picric or hydrochloric acid.

Renal clearance of endogenous creatinine is related to GFR and is normally 1 to 2 g/day (creatinine coefficient = 20 to 26 mg/kg/24 hr). Normal serum creatinine is 1 to 2 mg/100 mL; creatine 0.2 to 1.0 mg/100 mL. Higher values (5 mg/100 mL) indicate glomerular damage or cardiac insufficiency.

Uric acid is a catabolite of purine metabolism as derived from nucleic acids or nucleotide cofactors. Direct methods for determining uric acid involve the reaction with alkaline phosphotungstic acid to form a "tungsten blue," which is estimated colorimetrically. In another method, alcoholic NaOH is added to a protein-free filtrate to eliminate interfering reducing substances (ascorbic acid, glutathione) prior to the reduction of uric acid with acid copper chelate to form a cupric chromogen complex.

In indirect procedures, uric acid is hydrolyzed by the enzyme uricase; the decrease in absorbance at 290 to 293 nm is a function of the initial concentrations of uric acid. The normal blood value is 1.5 to 6.0 mg/100 mL. It is elevated in renal disease, gout due to increased metabolic pools of uric acid and leukemia as a result of increased turnover of cellular nucleoprotein.

Amino acid determinations in blood are performed by conventional colorimetric ninhydrin techniques or reaction with alkaline β-naphthoquinone-4-sulfonate. Normal plasma values range from 3.9 to 7.8 mg/100 mL. A variety of metabolic disorders may be detected by analyzing for increased levels of specific amino acids in the urine or blood. Total urine amino acids are determined by formol titration; formaldehyde reacts with basic amino groups and thus permits subsequent titration of the acidic groups of the amino acids. Daily excretion of amino acid nitrogen ranges from 100 to 400 mg, constituting 1 to 2% of total urine nitrogen.

The identification and quantitation of specific amino acids in the blood and urine are accomplished by paper, thin-layer (TLC), column and ion-exchange chromatographic and electrophoretic separation of electrolytically desalted blood or urine samples. See Chapter 29.

Abnormal amino acid metabolism (*aminoacidopathies*) usually results in the presence of abnormal quantities of specific amino acids in the urine (aminoaciduria).

The aminoacidurias are divided into two main groups:

1. *Primary overflow aminoaciduria* in which blood amino acids are elevated [phenylketonuria (PKU), maple syrup urine disease (MSUD), tyrosinosis and alkaptonuria].
2. Aminoacidurias characterized by elevated amino acid urine levels with normal blood levels (*transport diseases* with a defect in the kidney tubule—eg, cystinuria—and "no-threshold" aminoaciduria in which the kidney has no mechanism for reabsorbing the amino acid involved—eg, homocystinuria).

PKU, a disease characterized by mental deficiency, is associated with the presence of phenylpyruvic acid in the urine and elevated serum phenylalanine levels due to a hereditary (autosomal recessive) deficiency of hepatic phenylalanine hydroxylase, which converts phenylalanine to tyrosine. The availability of treatment through dietary intake is predicated upon early detection. Many states have passed legislation for mass-screening for PKU in all infants. The Guthrie test is performed by placing filter paper discs impregnated with serum or blood on the surface of an agar culture medium containing β-(2-thienyl)alanine at a concentration sufficient to inhibit the growth of *B subtilis*. *Phenylalanine* will reverse this inhibition and the Bacterial Inhibition Assay (BIA) is a direct measure of this amino acid. Serum phenylalanine determinations also can be performed by estimating the fluorescence of a complex with ninhydrin and copper in the presence of L-leucyl-L-alanine.

MSUD is characterized by the odor of the urine and rapidly is fatal to infants. It is associated with a deficiency in the oxidative decarboxylation of α-keto acids leading to an accumulation of both the keto and amino acids in the blood and urine (valine, leucine, isoleucine). TLC and BIA assays can be used to detect MSUD.

Alkaptonuria is a rare, hereditary disease in which homogentisic acid cannot be metabolized further due to a lack of homogentisic acid oxidase. This causes homogentisic aciduria, ochronosis and arthritis.

In *Hartnup disease*, indole and tryptophane appear in the urine due to defective renal and intestinal absorption of tryptophane. Tryptophane is an intermediary metabolite in the synthesis of serotonin (5-hydroxytryptamine) and 5-hydroxyindole acetic acid (HIAA). Excessive production of *serotonin* and the presence of its *HIAA metabolite* in the urine are associated with metastatic carcinoid tumors. HIAA is measured after removal of interfering keto acids with dinitrophenylhydrazine, extraction and estimation with nitrosonaphthol reagent.

Routine screening tests for congenital metabolic defects and the substance under test in the newborn include PKU (phenylalanine), MSUD (leucine), tyrosinemia (tyrosine), homocystinuria (methionine), histidemia (histidine), valinemia (valine), galactosemia (galactose or galactose uridyltransferase), orotic aciduria (orotidine-1-phosphate decarboxylase), arginosuccinuria (arginosuccinic lyase), hereditary angioneurotic edema (C^1-1-esterase inhibitor) and sickle-cell disease (hemoglobin S).

The analyses for these substances are based on BIA, metabolite bacterial inhibition assay (MIA), enzyme auxotraph bacterial assay (ENZ-Aux), fluorescent spot tests or TLC and electrophoresis.

Proteins—The *plasma proteins* (albumins, globulins and fibrinogen) are involved in nutrition, electrolyte and acid-base balance, transport mechanisms, coagulation, immunity and enzymatic action. *Total plasma proteins* may be determined by Kjeldahl, Nesslerization, specific ion-pair (bromcresol green dye plus albumin) or biuret procedures. The last technique is based on the reaction of —CONH— groups joined by carbon or nitrogen linkages in protein with alkaline copper sulfate to yield the biuret complex which can be estimated colorimetrically. Total protein also can be estimated by specific gravity, refractometric or UV spectrometric methods. These methods are subject to large errors in the presence of a pathology involving increased glucose, lipid, urea or abnormal protein concentrations.

The *albumin-globulin* (*A/G*) *ratio* is determined by the biuret method after precipitation of the globulins with a sodium sulfate-sulfite reagent. The normal range is 5.5 to 8.0 g% total protein with an A/G ratio of 1.4 to 2.4. Changes in total protein and A/G ratio occur in kidney and liver disease, hemorrhage, dehydration, rheumatoid arthritis and multiple myeloma. Gastrointestinal albumin loss, as seen in GI bleeding, ulcerative colitis, sprue and enteritis, can be

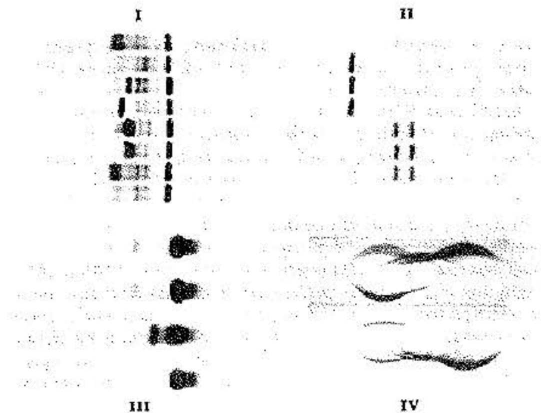

Fig 28-4. Electrophoretic separation of serum proteins (I), isoenzymes (II), hemoglobins (III), and immunoelectrophoresis of plasma protein (IV) (courtesy, Spinco).

detected by monitoring fecal radioactivity after IV injection of ^{51}Cr-human serum albumin.

The physiochemical properties of the plasma proteins—mol wt (68,000 to 300,000) and isoelectric point (pH of minimum solubility and ionic neutrality)—provide the basis for the electrophoretic separation of plasma proteins (Fig 28-4). The plasma sample is spotted on a paper or cellulose acetate strip, or in a polyacrylamide gel (disc or gel electrophoresis) at pH 8.6.

At this pH the proteins are electroanionic and, under the influence of electric current, will migrate to the anode at a rate dependent on their isoelectric point and, in the case of cellulose acetate or gel electrophoresis, their molecular size. The strips are then stained with a protein dye (bromophenol blue, Amidoschwarz or Ponceau S), and the concentrations of the various proteins are estimated by densiometric scanning.

The normal ranges for the major proteins are (in g%): albumin 3.8 to 5.0; total globulin, 2.0 to 3.9; α_1-globulin, 0.1 to 0.5; α_2-globulin, 0.5 to 0.9; β-globulin; 0.5 to 1.2; γ-globulin, 0.7 to 1.6.

Ordinary electrophoresis does not identify the subgroups of *immunoglobulins*, IgA, IgM, IgG and IgE. This is accomplished by immunoelectrophoresis, a process involving electrophoresis and immunodiffusion. The sample is electrophorized in an agar gel (zone electrophoresis) and then antiserum to the specific Ig or to total globulins is placed in a trough aligned parallel to the axis of the original electrophoresis. The serum proteins and antisera diffuse toward each other and form precipitin (antigen-antibody complex) lines. Ordinary cellulose acetate or gel electrophoresis will permit the recognition of diffuse, polyclonal elevation of serum immunoglobulins seen in chronic infections, isolated M-protein peaks of macroglobulinemia and multiple myeloma and absent gamma component in a hypogammaglobulinemia or agammaglobulinemia. Immunoelectrophoresis will indicate specific Ig abnormalities or, by noting the presence of any displacement, bowing or broadening of the precipitin band will aid in the diagnosis of the paraimmunoglobulin monoclonal diseases such as multiple myeloma, macroglobulinemia or chronic lymphatic leukemia.

Radial immunodiffusion is a simple process which also can be used for quantitation of IgA, IgM and IgG.[16] It is performed by incorporating the antibody in an agar gel and then introducing the antigen or test sera into wells punched in the agar. The antigen diffuses radially out of the well into the surrounding gel media, and a visible precipitin line forms where the antigen and antibody have reacted. Quantitation of IgA, IgM and IgG aids in the diagnosis and differentiation of collagen diseases, chronic infections and liver disease. IgE is best quantitated by immunoelectrophoresis or RIA (see section on *Immunology* for the basis and principles of RIA).

Nephelometric techniques detect immunological constituents by measuring the light-scattering properties of various antigen-antibody complexes in a test solution. The Hyland system measures the amount of laser-beam deflection at an angle by employing a photomultiplier tube which is sensitive in the red region of the spectrum. Results are calculated by an electronic-screening system and read in percent relative light-scatter on a digital readout.

Automated electrophoresis equipment offers computer-controlled sample application, staining options, densitometry and pattern interpretation for serum proteins and isoenzymes.

Enzymes—Enzymes are proteins whose biological function is the catalysis of chemical reactions in living systems. Enzymes combine with the substances on which they act (substrates) to form an intermediate enzyme-substrate complex which is then converted to a reaction product and liberated enzyme, which continues its catalytic function. Enzymes are highly specific; a few exhibit absolute specificity and catalyze only one particular reaction, while others are specific for a particular type of chemical bond, functional group or stereoisomeric structure.

Most serum enzymes of clinical significance are intracellular in origin and are elevated in hyperactivity disease, malignancy or injury to cardiac, hepatic, pancreatic, muscle, bone and tissue. As the specific tissue involved will determine the type of enzyme that will be elevated, such determinations are valuable diagnostic tools in the differentiation of various pathological states.

Enzymes are named and classified according to the type of reaction that they catalyze, and to their substrate specificities. Enzyme activity usually is expressed in International Units (IU) where 1 unit (U) is that amount of the enzyme which will catalyze the transformation of 1 μmole of substrate/min at definite temperature, pH and substrate-concentration conditions. Refer to Chapter 52 for a more complete discussion of enzymes.

Transferases are enzymes that catalyze the transfer of amino or phosphate groups from one compound to another. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are important in clinical diagnosis. These enzymes catalyze the transfer of the amino group from glutamic acid to keto acids (oxaloacetic or pyruvic) to form aspartic and α-ketoglutaric acids with AST (aspartate aminotransferase) and alanine and α-ketoglutaric acid with ALT (alanine aminotransferase).

Colorimetric methods are based on an estimation of the reaction products (oxaloacetic or pyruvic acid) with dinitrophenylhydrazine, or substrate (α-ketoglutaric acid) by coupling with 6-benzamido-4-methoxy-*m*-toluidinediazonium chloride.

Spectrometric methods are based on the reaction of the product pyruvate with lactic dehydrogenase and NADH, or of oxaloacetate with malic dehydrogenase and NADH. The rate of NADH utilization is measured by the decrease in absorbance at 340 or 360 nm and is directly proportional to transaminase activity.

Normal AST and ALT levels are <40 mU/mL. AST is present in large amounts in liver, cardiac and skeletal muscle, whereas ALT is found primarily in liver tissue. AST is elevated in myocardial infarction and Duchenne muscular dystrophy; AST and ALT are increased in liver disease, acute toxic or viral hepatitis, infectious mononucleosis, obstructive jaundice and hepatic cirrhosis.

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Creatine kinase (*CK*) is a transferase found in muscle and brain tissue. It catalyzes the transfer of phosphate groups from creatine phosphate to adenosine diphosphate (ADP) to form adenosine triphosphate (ATP). Activated CK activity is measured by following the increase of ATP in the creatinine phosphate-ADP reaction in the presence of glutathione or cysteine thiol activators. The ATP can be measured by the fluorimetric determination of light emitted by luciferinase conversion of luciferin to adenyl-oxyluciferin in the presence of ATP. Normal serum levels are <50 mU/mL; it is elevated in myocardial infarction and Duchenne muscular dystrophy, but remains at normal levels in liver disease.

Ornithine transcarbamylase (*OTC*) in serum is the only enzyme of the urea cycle which has been used in the clinical investigation of liver disease. It catalyzes the conversion of ornithine to citrulline. The normal serum value is 0 to 0.4 mU/mL.

Oxidoreductases or *dehydrogenases* are enzymes that catalyze hydrogen transfer in cellular oxidation processes. *Lactic* (*LDH*), *α-hydroxybutyric* (*HBDH*), *malic* (*MDH*), *glutamic* (*GLDH*), *isocitric* (*ICDH*) *and sorbitol* (*SDH*) *dehydrogenases* are of diagnostic importance in myocardial and liver disease.

LDH catalyzes the reversible conversion of pyruvic to lactic acid in the presence of NADH. The activity may be estimated colorimetrically by forming the pyruvic acid hydrazone with 2,4-dinitrophenylhydrazine; spectrometric or fluorimetric estimation of NADH in this reaction also is used to estimate enzyme activity. The normal serum LDH value is <200 mU/mL (pyruvate → lactate) and <50 mU/mL (lactate → pyruvate). LDH is increased to a much greater extent and for a more prolonged period than AST or CK in myocardial infarction; it also is increased to varying degrees in certain types of hepatic disease, disseminated malignancies, pernicious anemia and muscular dystrophy.

Recent advances in protein chemistry and technical methodology have led to fractionation of enzymes, previously thought to be homogeneous, into heterogeneous moieties. These multiple-molecular forms of enzymes (*isoenzymes*) have similar substrate specificity but different biophysical properties. LDH, MDH, CK, phosphatases and leucine aminopeptidase exist in isoenzyme forms.

CK isoenzymes are important in the early detection of myocardial damage. Two CK molecular subunits, M and B, produce three isoenzymes: CK-MM found primarily in skeletal muscles, CK-MB in the myocardium and CK-BB primarily from the brain. After acute myocardial infarction (MI), CK-MB appears in the serum in approximately 4 to 6 hours, reaches peak activity at 18 to 24 hours and may disappear within 72 hours. Diagnostic testing of MI includes CK and LDH isoenzymes. Early detection of CK-MB allows the management of myocardial infarcts with agents such as streptokinase or tissue plasminogen activator (TPA). The methods of assessment include electrophoresis, column chromatography and immunoinhibition.

Serum contains five LDH isoenzymes, each a tetramer composed of one or two monomers. LDH 1 and 2 are found in preponderance in heart, kidney and RBC; whereas liver and skeletal muscle largely contain LDH 4 and 5. Intermediate forms prevail in lymphatic tissues and many malignancies. The fractionation of LDH isoenzymes is important in the differential diagnosis of cardiac, muscle and liver disease. It can be accomplished with DEAE-cellulose chromatography, electrophoresis, sulfite or urea inhibition of specific isoenzymes, thermal stability and substrate-concentration requirements.

HBDH reduces α-ketobutyric acid to α-hydroxybutyric acid in the presence of NADH; estimation of the α-keto acid via hydrazone formation or NADH is the basis of activity measurements. The normal serum HBD level is <140 mU/mL; it is elevated in myocardial infarction. LDH 1 is high in HBDH activity. The ratio of total LDH/HBDH often is used in place of LDH isoenzyme determination. Ratios >0.8 are seen in myocardial infarction and <0.6 in acute liver damage.

MDH and *SDH*, in the presence of NAD, catalyze the conversion of malate or sorbitol to oxaloacetate or fructose, respectively. They are of diagnostic value in MI (MDH >48 mU/mL) and acute liver injury (SDH >96 mU/mL).

ICDH oxidizes isocitrate, in the presence of NADP or NAD, to α-ketoglutarate; it is elevated (>5.0 mU/mL) in acute hepatitis.

Hydrolases are enzymes that catalyze the addition of the elements of water across the bond which is cleaved. *Amylases, lipases, phosphatases, 5′-nucleotidase, γ-glutamyl-transpeptidase* and *leucine aminopeptidase* are specific examples of clinically important hydrolases.

Salivary and pancreatic *amylases* hydrolyze the substrate starch to maltose and dextrins. Amylase activity can be measured by procedures based on the loss in certain properties of starch as it is hydrolyzed (*amyloclastic*), or by the generation of reducing substances (*saccharogenic*). The amyloclastic methods use the decrease in viscosity and turbidity of hydrolyzed water-soluble starch substrates, or the reaction of starch with iodine as the method of estimation. A newer procedure uses the colorimetric determination of water-soluble dye-dextrin fragments released by amylolytic hydrolysis of a cross-linked, water-insoluble, dye-starch polymer. The saccharogenic methods determine the reaction products (reducing sugars) by a previously described methodology. The normal serum level is 140 mU/mL; elevations are noted in acute pancreatitis, acute abdominal conditions (perforated peptic ulcer, common bile-duct obstruction) and salivary gland disease.

Lipases catalyze the conversion of triglycerides to glycerol and fatty acids. Clinical determinations are based on the titrimetric analysis of fatty acids liberated from an emulsified olive oil substrate, or fluorimetric estimation of fluorescein liberated from a fluorescein fatty acid ester substrate. Serum lipase is increased in pancreatic carcinoma.

Phosphatases catalyze the hydrolysis of orthophosphoric acid esters, and are classified according to the pH of optimal activity into alkaline or acid phosphatases. Activity (alkaline, pH 8 to 10; acid, pH 4 to 6) is measured with phenyl phosphate, glycerophosphate, *p*-nitrophenyl phosphate or thymolphthalein monophosphate substrates. With the latter two chromogenic substrates, the amount of *p*-nitrophenol or thymolphthalein liberated by phosphatase hydrolysis is estimated colorimetrically in an alkaline medium. With a glycerophosphate or phenyl phosphate substrate, the liberated phosphorus is determined by molybdenum blue formation with phosphomolybdic-phosphotungstic acids; phenol also may be estimated with 4-aminoantipyrine or Folin-Ciocalteau reagent.

Acid phosphatase activity may be differentiated by the use of inhibitors in the assay mixture; formaldehyde has no effect on acid phosphatase of prostatic origin, but it inhibits other acid phosphatases, while tartrate is a selective inhibitor of the prostatic enzyme. *Acid phosphatase* is of a primary diagnostic value in metastatic carcinoma of the prostate.

Normal values for *alkaline phosphatase* activity depend on the substrate used; elevations in osteomalacia and in bone tumors depend on the degree of osteolytic or osteoblastic activity. The enzyme (isoenzyme) also is elevated in obstructive jaundice, bone and liver disease.

The enzyme *5′-nucleotidase* is an alkaline phosphomonoesterase that hydrolyzes nucleotides with a phosphate radical attached to the 5′-position of the pentose (eg, adenosine monophosphate). The normal serum value is 17 mU/mL; it is elevated in hepatic disease.

Leucine aminopeptidase (LAP) is an exopeptidase which

hydrolyzes the peptide bond adjacent to a free amino group. It liberates amino acids from the *N*-terminal group of proteins and polypeptides in which the free amino group is a L-leucine residue. Activity is determined by spectrophotometric estimation following hydrolysis of the amide bond of a leucinamide substrate at 238 nm. Clinical estimations usually are performed on synthetic substrates, and since there is no correlation between cleavage of leucinamide and these substrates, the LAP-like activity is designated *leucine arylamidase*. A fluorometric determination of naphthylamine liberated from a leucyl-β-naphthylamide substrate or colorimetric determination of *p*-nitroaniline liberated from leucino-*p*-nitroanilide substrate also has been used. The normal value is 8 to 22 mU/mL; it is elevated in the last trimester of pregnancy, hepato-biliary disease and pancreatic carcinoma.

Serum *γ-glutamyl transpeptidase* (γGT) is increased in diseases of the liver, bile ducts and pancreas. Together with alkaline phosphatase, LAP and 5′-nucleotidase, γGT usually is tested in the group of cholestasis-indicating enzymes. The assay is based on the hydrolysis of γ-glutamyl-*p*-nitroanilide.

Serum lysozyme (muramidase) activity is increased in certain types of leukemia. Serum arginase, an enzyme which hydrolyzes arginine to ornithine and urea, and serum guanase are sensitive indicators of hepatic necrosis.

Lyases are enzymes which split C—C bonds without group transfer. *Aldolase* is a glycolic lyase which catalyzes the reversible splitting of fructose 1,6-diphosphate to form dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. In the estimation of activity, the triose phosphate reaction products are hydrolyzed with alkali and the resultant trioses are reacted with 2,4-dinitrophenylhydrazine to form chromogenic hydrazones for colorimetric analysis. A spectrophotometric estimation is made by coupling the aldolase reaction products with a dehydrogenase acting on one of the triose phosphates and measuring concomitant changes in NADH. The normal value is <8 mU/mL; it is elevated in muscular dystrophy, polymyositis and acute hepatitis.

The significance of serum-enzyme changes in hepatitis is seen in Fig 28-5 and enzyme activity following myocardial infarction in Fig 28-6.

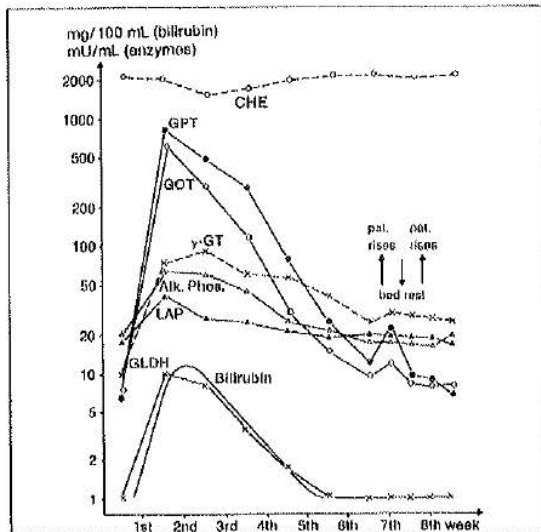

Fig 28-5. Typical course of alterations in serum enzyme activity in acute viral hepatitis (courtesy, Schmidt E, Schmidt FW *Med Welt 21*: 805, 1970).

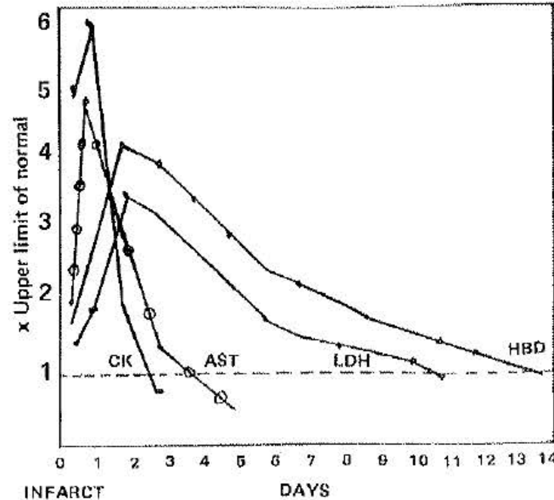

Fig 28-6. Serum enzymes following myocardial infarction, AST, CK, LDH and HBD are compared.

Lipids—The major classes of blood lipids are *fatty acids, cholesterol, triglycerides, phospholipids and lipoproteins.* Hyperlipidemia is not a single aberration and there are a number of different hyperlipidemic states. Lipid-profile tests include measurements of cholesterol, triglyceride, phospholipids and determination of lipoprotein phenotypes.

Cholesterol, a sterol molecule, is an essential substance in steroid-hormone synthesis by the adrenal cortex and bile acid production in the liver. It exists in blood as the free sterol and as cholesterol esters of fatty acids.

In the determination of *total cholesterol*, the serum is extracted with an alcohol-ether mixture and the cholesterol estimated colorimetrically after reaction with acetic anhydride-sulfuric acid reagent (Liebermann-Burchard reaction). The precipitation of free cholesterol with digitonin will differentiate free from esterified cholesterol. Chromatographic separation of cholesterol from its esters on alumina, silicic acid or magnesium silicate columns with organic solvents also has been used.

Gas chromatographic procedures have resulted in the separation and quantitation of cholesterol, its metabolites and precursors; this is a type of partition chromatography in which a volatilized sample is partitioned between a liquid stationary phase and a mobile gas phase. The normal-adult total-serum-cholesterol level is 150 to 270 mg/100 mL; it is increased in hyperlipemia and specifically in hyper-β-lipoproteinemia, nephrosis, diabetes mellitus and myxedema, and decreased in hyperthyroidism and hepatic disease. Free cholesterol comprises 20 to 40% and the ester fraction 60 to 80% of the total serum cholesterol.

Phospholipids are "compound" or "heterolipids" which contain phosphorus, a nitrogen base and a long-chain fatty acid. Lecithin (phosphatidylcholines) and cephalin (phosphatidylethanolamine or serine) are the principal plasma phospholipids, which normally comprise one-third of the total plasma lipids. They usually are bound to lipoproteins. These serum lipids are extracted into an alcohol-ether mixture, digested with sulfuric acid-hydrogen peroxide and the liberated phosphorus determined by colorimetric techniques. The normal lipid phosphorus is 6 to 11 mg/100 mL; about one-half is lecithin. The average ratio of cholesterol to lipid phosphorus when cholesterol is normal is 21. Phospholipid changes usually are associated with cholesterol changes and are of interest in coronary artery and liver diseases and the hyperlipoproteinemias.