Zeta Potential in the Development of Pharmaceutical Suspensions

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A discussion of a microelectrophoretic mobility apparatus (Zeta-Meter) is presented in connection with some prerequisite studies with a model dispersed system. The limitations of the fundamental Helmholtz-Smoluchowski formula for calculating zeta potential as it applies to a study of real pharmaceutical systems is presented. A description of the apparatus, including a discussion of the advantages of the dark-field stereoscopic microscope, molybdenum-platinum electrode system, cylindrical, tube-type, plastic electrophoresis cell, and the automatic sample-transfer device is also presented. The usefulness of zeta potential in developing pharmaceutically stable, flocculated suspensions is illustrated with three different examples: (*a*) an adrenocorticoid in which flocculation was directly achieved by varying the concentration of electrolyte added; (*b*) a system in which flocculation was initially induced in the absence of an electrolyte; (*c*) the development of a diagnostic system for blood-cell agglutination testing based upon the principles of zeta potential.

ALTHOUGH the zeta potential (ZP) concept as it relates to particles in suspension has been known to colloid chemists for more than 40 years (1–3), only recently, with the introduction of new techniques for measuring electrophoretic mobility (EM), have industrial and pharmaceutical applications of its principles been possible (4–6, 15). Haines and Martin (7), studying the electrokinetic behavior of insoluble drug particles in dispersion, were able to correlate the magnitude of ZP to the caking tendencies of pharmaceutical suspensions. Since then little has been reported in the pharmaceutical literature concerning ZP. Even though the debate over the physicochemical meaning of ZP continues in the general literature (8,9), the electrokinetic behavior of dispersed systems can still be investigated in terms of an observable quantity, the EM value.

The processes involved in the formation of pharmaceutical suspensions and the concept of ZP as it applies to a study of such systems have been reviewed recently (10).

The ZP of a particle in suspension can be calculated approximately from EM with the aid of the simple Helmholtz-Smoluchowski Eq. 1. Where ZP represents the zeta potential of the suspensoid in millivolts, 4π is the Smoluchowski factor, the factor 9×10^3 converts electrostatic units to practical electrical units and microns to centimeters, η is the viscosity of the suspending liquid in poise, ϵ represents the dielectric constant of the suspending liquid, and EM represents the electrophoretic mobility of the suspensoid in μ/sec./v./cm.

$$ZP = 4\pi \cdot 9 \times 10^3 \cdot \frac{\eta}{\epsilon} \cdot EM \qquad \text{(Eq. 1)}$$

For suspensions in water at 25° Eq. 1 is reduced to

$$ZP = 12.869 \cdot EM \qquad \text{(Eq. 2)}$$

Calculation of so-called "true ZP values" in terms of the physical meaning of the system under study involves refinement of the 4π multiplication factor (9). Its magnitude, which is related to the thickness of the electrical double layer surrounding the particle, is dependent upon particle size and shape, the type and concentration of electrolyte in the bulk liquid, the distortion of the electric field by the suspended particle (electrophoretic retardation effect), the surface conductance of the electrical double layer, and ZP itself.

A series of multiplication factors for systems containing a typical 1:1 electrolyte (potassium chloride), which takes into account the influence of some of these systematic variables, have been calculated and summarized in Table I. An inspection of these values reveals that when the size of the electrical double layer is small with respect to the particle, *i.e.*, in suspensions with moderate to high concentrations of electrolyte, the Smoluchowski 4π factor can be employed with sufficient accuracy.

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TABLE I.—FACTORS FOR A 1:1 ELECTROLYTE TO BE USED IN THE HELMHOLTZ-SMOLUCHOWSKI FORMULA IN PLACE OF THE 4π FACTOR

KCl Concn., % w/v	Specific Conductance, μmhos./cm.	κa [a]	ZP, mv. 0	25	50	70
		a, Particle Radius = 1 μ				
1.0×10^{0}	1.775×10^{4}	1203.	4.00π	4.00π	4.00π	4.00π
1.0×10^{-1}	1.820×10^{3}	380.	4.04π	4.08π	4.08π	4.12π
1.0×10^{-2}	1.905×10^{2}	120.3	4.16π	4.20π	4.24π	4.32π
1.0×10^{-3}	2.04×10^{1}	38.	4.40π	4.48π	4.64π	4.88π
1.0×10^{-4}	2.2×10^{0}	12.	4.84π	5.00π	5.36π	6.12π
		a, Particle Radius, μ	0	25	50	70
1.0×10^{-3}		1.0	4.40π	4.48π	4.64π	4.88π
1.0×10^{-3}		2.0	4.20π	4.24π	4.39π	4.52π
1.0×10^{-3}		4.0	4.12π	4.16π	4.16π	4.28π

[a] κa represents the ratio between the radius of the particle and the extension of the electrical double layer.

DISCUSSION

However, when the electrical double layer is extended, *i.e.*, in suspensions which contain little or no electrolyte, then the factor approaches Henry's 6π value (11). It is also well to point out that the concentration of ions (charge density) in the electrical double layer bears an inverse relationship to the size of the double layer itself.

The use of the Helmholtz-Smoluchowski formula is further complicated by the fact that the viscosity term in Eq. 1 exerts a more pronounced effect upon ZP than does the dielectric constant. For example, in calculating the ZP for particles dispersed in 50% syrup (sucrose in water) where the viscosity of the suspending vehicle at 25° is 0.125 poise and the dielectric constant is 64.2, the EM of the particle would have to be reduced by a factor of $^{1}/_{17}$ to give a ZP equivalent to that in water at the same temperature. The reason is that the viscosity of syrup is 14 times greater than that of water, while the ϵ of syrup is only $^{4}/_{5}$ of the value for water. With water systems thickened with a small amount of a hydrophilic colloid such as carboxymethylcellulose U.S.P. or methylcellulose U.S.P. (where the ϵ is essentially equal to that of water) the viscosity effect would be even greater. Therefore, it would seem plausible that the η term in Eq. 1, which in essence represents the shear stress between the electrical double layer and the bulk of the liquid, is only applicable to simple liquids. The use of non-Newtonian liquids and vehicles with viscosities greater than several centipoise units will seriously limit the usefulness of the basic Helmholtz-Smoluchowski expression.

EM is equal to the particle velocity per unit field strength and is expressed in μ/sec./v./cm. Particle velocity is determined microscopically by multiplying the divisions traveled per μ per eyepiece division and dividing the result by the time traveled in seconds. If a circular tube-type electrophoresis cell is employed, then the circle of zero endosmotic flow is situated at a depth which is 14.7% of the tube diameter. Since the over-all flow of the suspending liquid is zero at this point, the net motion is that of the particles themselves.[1] EM can be calculated from either the effective length of the electrophoresis cell, *l*, in cm.

[1] The electrophoresis cell of the apparatus is so designed as to make this critical depth coincide with the counting line on the eyepiece of the stereoscopic microscope.

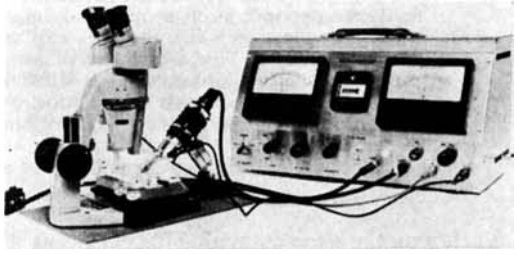

Fig. 1.—The instrument for measuring electrophoretic mobility of drug particles in suspension. Detailed instruction for its operation and calibration are provided with the equipment.

$$\text{EM} = \frac{v \cdot l}{E} \qquad \text{(Eq. 3)}$$

or from the cross-sectional area of the cell, *q*, in cm.^{2},

$$\text{EM} = \frac{v \cdot q \cdot L}{I} \qquad \text{(Eq. 4)}$$

where *v* equals particle velocity in μ/sec., *E* is the applied voltage in practical volts, *L* is the specific conductance of the cell, and *I* is the current flow through the cell in amperes. In the authors' work both Eqs. 3 and 4 were used to calculate EM.

APPARATUS

A new device[2] measures the microelectrophoretic mobility of particles in aqueous suspension. The term "Zeta-Meter" is a misnomer since the instrument neither measures nor determines directly the ZP or effective surface charge of dispersed particles. The equipment is shown in Fig. 1.

The apparatus offers the following advantages.

(*a*) Availability of a ready-to-use complete research system for determining the EM values of colloids and small dispersed particles in essentially aqueous suspension.[3]

(*b*) Since the anode chamber of the Zeta-Meter electrophoresis cell is closed and the cathode chamber is open, when electric current passes through

[2] Zeta-Meter. Marketed by Zeta-Meter, Inc., New York, N. Y.

[3] The authors are unable to comment about the capability of the instrument with respect to its use in connection with essentially nonaqueous systems because of their lack of familiarity with this particular field of study.

systems with a high concentration of electrolyte, appreciable amounts of oxygen can be produced at the anode, due to electrolysis. Such gas formation will interfere with the performance of the cell by inducing liquid movement and false particle migration. The use of a readily oxidizable molybdenum anode, which forms a tight film of oxide on its surface, prevents gaseous imbalance from creating a condition of liquid flow in the cell. A platinum strip cathode, at which hydrogen is evolved, does not induce false migration as the gas passes freely into the atmosphere. However, at low electrolyte concentrations, electrolysis is not a problem and a pair of platinum electrodes can be used. The solid molybdenum-platinum electrodes, which are provided with the unit, are more convenient than a solid-liquid, copper-saturate copper sulfate coupling, with porous plaster of paris plugs which have been previously employed to prevent gasification (12).

(*c*) The use of a stereoscopic microscope of sufficient magnification (120 to 180×) with bounced-beam illumination permits indirect viewing of colloids below 1 μ by reflected light emanating from the particle's surface. The technique is commonly referred to as the "dark-field effect." Effective view of particles extends from 0.05 to 0.02 μ. However, the lower limit of view depends upon particle shape.

(*d*) The cylindrical, tube-type plastic electrophoresis cell utilized offers several obvious advantages over the conventional Briggs flat thin-glass cell which for years has been considered a standard for EM measurement (13). The over-all depth of a Briggs cell (0.6 to 1.0 mm.) limits its ability to handle particle flocs greater than 0.1 mm. Such suspensions can be measured quite easily with the Zeta-Meter cell. In addition, the rugged construction of the plastic cell is in sharp contrast to the fragility of the glass Briggs cell.

(*e*) A new automatic sample-transfer device, also available with the basic unit, is shown in Fig. 2. This device rapidly clears the cell reintroducing fresh sample between measurements, and thereby eliminates the problem of particle drift due to the convection currents by maintaining a condition of isothermality in the cell. The problem of particle drift and thermal overturn is especially acute in flocculated or partially flocculated systems where EM is extremely slow and electrolyte concentration is high. In addition, the use of this auxiliary equipment permits an increase in the number of EM measurements that can be made with the basic unit, thereby enabling the equipment to be used as an EM or ZP titrimeter for particle adsorption and desorption study.

(*f*) In addition to these basic features, the electrophoresis cell can function as a conductivity cell once it is calibrated in the usual manner with a standard potassium chloride solution. Specific conductance values thus obtained are of sufficient accuracy and can be determined during the course of an EM measurement.

However, Schmut (14) lists several disadvantages for the apparatus in a paper concerning ZP applications in the paper industry. The most serious drawback he found is its sensitivity to vibration. The problem is especially acute when the electrophoresis cell is connected to the automatic sample-transfer device. Nevertheless, the condition can be alleviated somewhat by the use of coarse nylon screens in the inlet and outlet tubes leading to the cell. The screen apparently disrupts eddy currents that are set in motion by the oscillation of the automatic pinch-clamp.

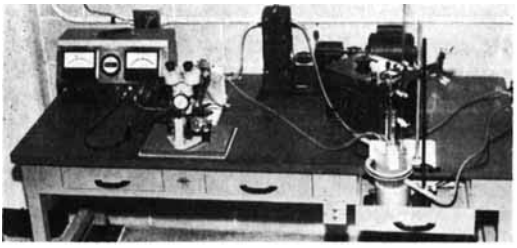

Fig. 2.—Instrumentation required to perform EM or ZP titrations, including the automatic sample-transfer device, Beckman model G pH-meter, and Zeta-Meter.

The authors have found that poor reproducibility of experimental results and meaningless EM values can be attributed to the limitation of the ZP theory to explain the significance of such data rather than instrument failure itself. Furthermore, problems of particle settling during measurement and false migration in difficult systems can be greatly diminished by the use of the automatic sample-transfer device.

STUDIES WITH PURIFIED SILICA

Purified silica[4] was selected as a model dispersed system for study. Such studies are important both as a prerequisite for work with more difficult pharmaceutical systems and to enable the investigator to become more familiar with the equipment and techniques involved in EM measurement. The material consists of high-purity, microcrystalline silica containing 99.9% silicon dioxide. Silica approaches an ideal solid for study since the material is relatively inert, suspensions of it are essentially neutral, and complications associated with surface ion-exchange are negligible (15). An additional advantage is that the material is easily wetted and simple aqueous dispersions can be conveniently prepared with low-shear equipment.

Dispersing Silica with Surfactants.—With a standard 0.01% suspension of purified silica in fresh nitrogen-sparged distilled water, a series of dispersion curves were developed with three typical surface-active agents (more commonly referred to as surfactants). They included an anionic surfactant (dioctyl sodium sulfosuccinate N.F.), a cationic surfactant (benzalkonium chloride U.S.P.), and a nonionic surfactant (polysorbate 80 U.S.P.). Surfactants are primarily used as wetting agents for hydrophobic particles, which act in this capacity by lowering the interfacial tension between suspensoid and vehicle. They can, however, also function as dispersing agents, *e.g.*, by being adsorbed on the surface of silica particles. Adsorption of a negatively charged, polymeric anionic surfactant increases the negative ZP of dispersed suspensoid particles, while conversely, adsorption of a positively charged,

[4] Purified crystalline silica was supplied by the Pennsylvania Glass Sand Corp. under the trademark 5-μ Min-U-Sil; 98% of this material has a diameter less than 5 μ. The surface area of the sample is 20,600 cm.^{2}/Gm. with an average particle size of 1.1 μ.

cationic specie reduces the existing negative ZP on silica particles. Addition of more cationic surfactant eventually produces particles that are positively charged. Since in both instances these agents are ionic in nature, there are enough dissociated counterions in the bulk liquid to induce adsorption.

Adsorption curves showing the influence of an anionic surfactant (dioctyl sodium sulfosuccinate N.F.) and a cationic surfactant (benzalkonium chloride U.S.P.) on the ZP of a 0.01% purified silica dispersion are given in Fig. 3. ZP of the suspensoid apparently increases linearly as increasing amounts of dispersant (surfactant) are added to the system. A point of maximum particle dispersion is reached which corresponds to the point of maximum ZP, either negative or positive depending upon the surfactant under study. In an adsorption process (which will be briefly discussed later) this also corresponds to the formation of a monolayer of adsorbed surfactant. Further addition of surfactant beyond this critical point produces no additional change in ZP which is reflected in no further adsorption of dispersant. The region of maximum ZP for these dispersants is also apparently very close to the critical micelle concentration (CMC) of the surfactants themselves.[5] Since the shapes of interfacial tension curves for determining the CMC of surfactants are also quite similar to the dispersion curves shown in Fig. 3, and since both parameters are manifestations of a more basic adsorption process, it is conceivable that a ZP technique, such as the one described above, can also be used to determine the CMC of surfactants.

With nonionic surfactants (Fig. 4), since the polymer supplies no appreciable ionic species to induce adsorption, the addition of an electrolyte, such as potassium chloride, is required. In essence, the presence of an electrolyte alone is all that is required to disperse silica particles. The addition of a nonionic surfactant merely decreases the concentration of electrolyte required for dispersion. However, the presence of a nonionic surfactant such as polysorbate 80 U.S.P. also apparently decreases the ZP required for complete dispersion and thus interferes with the establishment of a strong electrical double layer, probably through steric hindrance of adsorbed, bulky, surfactant molecules. Therefore, nonionic surfactants are probably less efficient dispersing agents for silica and other suspensoids than are their ionic counterparts.

Influence of Various Polyvalent Anions on Purified Silica Dispersions.—As was previously pointed out, electrolytes alone can be used to disperse silica particles in suspension. A series of dispersion curves, in which the addition of various sodium salts (chloride, sulfate, citrate, and pyrophosphate) were used to disperse a standard 0.01% silica suspension, are shown in Fig. 5. The salts show an increasing order of dispersing efficiency as polyvalent anions. On a molar basis, approximately 3 times more citrate ion, approximately 6.6 times more sulfate ion, and 10.6 times more chloride ion is required to achieve the same degree of dispersion as with the quadrivalent pyrophosphate ion.

The magnitude of the ZP of dispersed silica particles is also increased when polyvalent ions are used to aid dispersion. In all cases, however, the addition of excess electrolyte well beyond the point of maximum dispersion causes a reduction in ZP which has been described as the "bulk stress effect" (17). The phenomenon is probably caused by the collapse of the electrical double layer by the proximity of large numbers of counterions in the vicinity of the particle. With particles that are coated with adsorbed colloids or polyelectrolytes, bulk stress may induce a "salting out" of colloid, which alone is sufficient to decrease ZP.

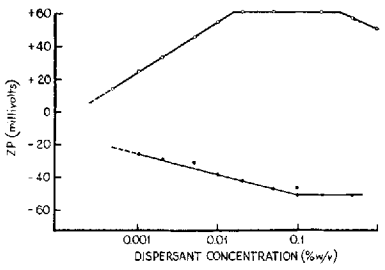

Fig. 3.—The influence of two ionic surfactants on the ZP of a 0.01% purified silica suspension. Key: top curve, benzalkonium chloride U.S.P. (cationic type); bottom curve, dioctyl sodium sulfosuccinate N.F. (anionic type).

Quantitative treatment of the efficiency with which polyvalent electrolytes disperse silica is complicated by the ionization, in solution, of the various polyprotic acids (sulfuric, citric, and pyrophosphoric) which produce these salts. No attempt was made in this study to work at a constant pH value, nor was there any attempt to consider the contribution of lower valence anions in the dispersion process.

In addition to their ability to increase the efficiency of particle dispersion, the size and type of both anions and cations are also important in the flocculation of suspended particles (18).

Dispersion as an Adsorption Process.—Another basic study of silica was concerned with determining the amount of dispersant required for maximum dispersion. Dispersion curves were constructed for a series of concentrated silica suspensions. Sodium hexametaphosphate was selected as a dispersing agent.[6] Increasing amounts of the dispersant were added to each suspension in order to determine, graphically, the amount of reagent required to reach the point of maximum ZP. This particular value is associated with the adsorption of a monolayer of dispersant on the silica particles. The amount of dispersant required in the bulk liquid to initiate adsorption was readily determined by preparing a very dilute 0.001% silica suspension. Since the concentration of silica was extremely small (10 p.p.m.) EM measurements were made directly on the suspension. In determining the EM values for the more concentrated silica slurries (5, 10, 20, and 50%), after a calculated amount of dispersant was added during each step in the construction of the dispersion curve, a sample of the slurry was filtered through a medium porosity, cellulose nitrate-membrane filter to obtain a clear filtrate of equilibrated bulk liquid. A small amount of concentrated residue was added to permit maximum visibility of silica particles for measurement. Samples prepared

[5] Determined experimentally by the surface tension method (16).

[6] The sodium hexametaphosphate used in this study was a sample supplied by the Olin Mathieson Chemical Corp. under the product designation Sodium Polyphos Ground.

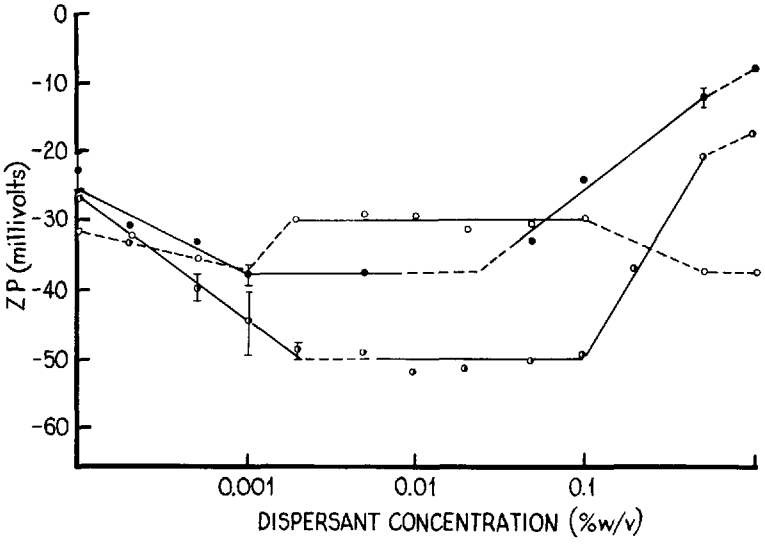

Fig. 4.—The influence of a nonionic surfactant on the ZP of a 0.01% purified silica suspension. Key: ○, polysorbate 80 (nonionic type); ●, polysorbate 80 + KCl; ◑, KCl (neutral electrolyte).

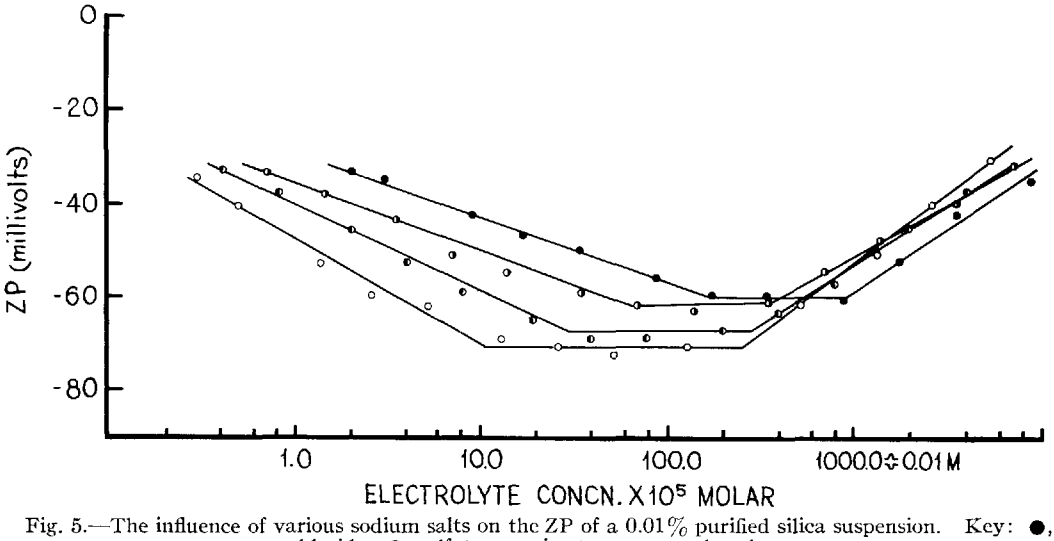

Fig. 5.—The influence of various sodium salts on the ZP of a 0.01% purified silica suspension. Key: ●, chloride; ◑, sulfate; ◐, citrate; ○, pyrophosphate.

in this manner gave EM values that were representative of the concentrated slurry from which they were prepared. In this manner, a series of dispersion curves were constructed for highly concentrated slurries of silica which could not be measured in the electrophoresis cell directly. The resulting dispersion curves are presented in Fig. 6.

The relationship between the amount of dispersant adsorbed and its concentration in the suspending vehicle may be represented by the adsorption equation of Langmuir (19), which is based upon a supposition that dispersion is fundamentally an adsorption process. A plot of concentration of dispersant per ZP *versus* concentration of dispersant yields a straight line whose slope is equal to some conversion constant multiplied by the Langmuir factor β/α and an intercept equal to $1/\alpha$. The Langmuir equation differs from other adsorption isotherms in that adsorption approaches a finite limit (the point of maximum ZP) as the concentration of dispersant added is increased. However, beyond the point of maximum dispersion no further adsorption takes place (Figs. 3–6). The relative constancy in ZP beyond the point of maximum dispersion is further proof that these particular dispersions conform to a Langmuir adsorption process.

In Fig. 6, the average amount of sodium hexametaphosphate adsorbed per gram of silica was found to be 0.655 mg./Gm. and the amount of dispersant in the bulk liquid required to initiate adsorption was 0.052 mg./ml. Since sodium hexametaphosphate is a water-soluble, glassy, condensed polymeric phosphate of indefinite composition, no attempt was made to determine the surface area of dispersant for monolayer adsorption. Nevertheless, the amount of dispersant required for complete disper-

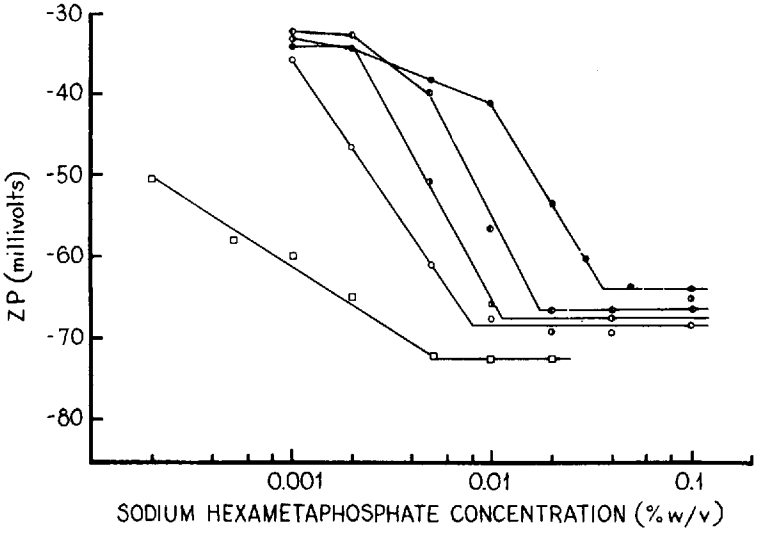

Fig. 6.—Dispersion curves for dilute and concentrated silica suspensions prepared with sodium hexametaphosphate. Key: □, 0.001% silica; ○, 5%; ◑, 10%; ◐, 20%; ●, 50%.

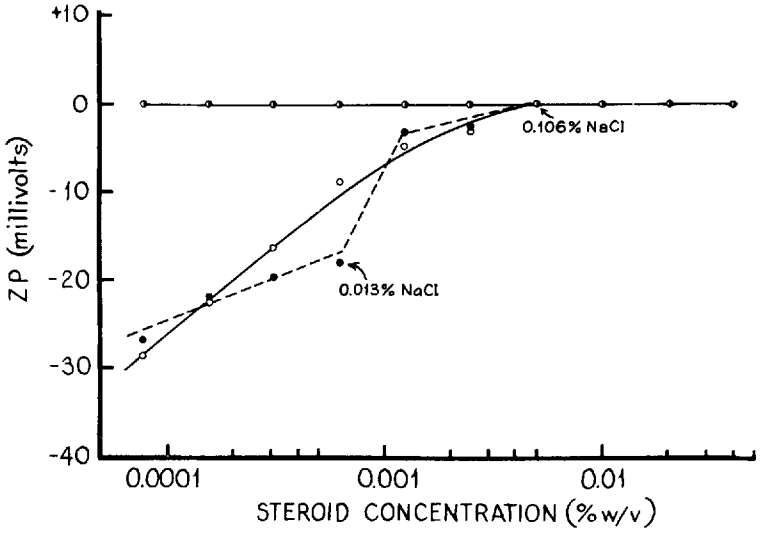

Fig. 7.—Desorption curves of a flocculated adrenocorticoid suspended in various partial vehicles. Key: ◑, partial vehicles containing NaCl; ●---●, diluted with salt-free vehicle. Curve shows differential desorption; ○—○, diluted with water.

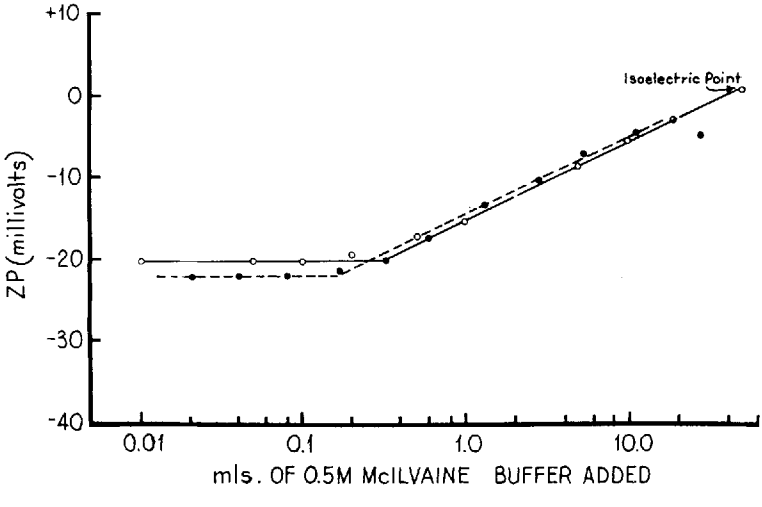

Fig. 8.—Flocculation curve for a drug suspension which was initially partially flocculated with nonelectrolytes. Key: - - - - -, desorption curve; ——, adsorption curve.

sion for any given slurry concentration of silica can be calculated according to the method of Riddick (20) from the two values reported above.

Attempts to determine the amount of an anionic or cationic surfactant required to completely disperse silica or any other suspensoid, are complicated by the fact that a portion of surfactant added to such systems usually is involved in micelle formation, which apparently is a competitive physical phenomenon.

APPLICATION OF ZETA POTENTIAL IN PHARMACEUTICAL PRODUCT DEVELOPMENT

The following three examples were chosen to illustrate how ZP can be used to develop pharmaceutically stable, flocculated suspensions.

Influence of Electrolyte in Altering ZP.—A formulation for a parenteral suspension of an adrenocorticoid was developed empirically without the use of the apparatus. The system consisted of 4% micronized steroid suspended in an aqueous vehicle composed of a wetting agent (polysorbate 80 U.S.P.) to disperse the drug, a preservative (benzyl alcohol), an electrolyte (sodium chloride) to adjust tonicity, and a small concentration of an adjuvant polymer (polyethylene glycol 4000 U.S.P.) to provide a pharmaceutically stable, "noncaking"

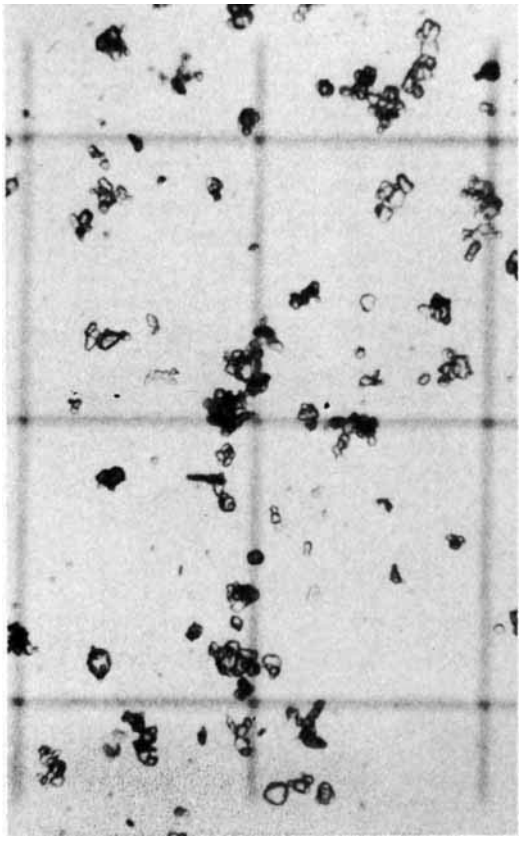

Fig. 9.—Photomicrograph of a deflocculated suspension prior to the addition of a critical concentration of a nonelectrolyte. The width of each scale division equals 96 μ.

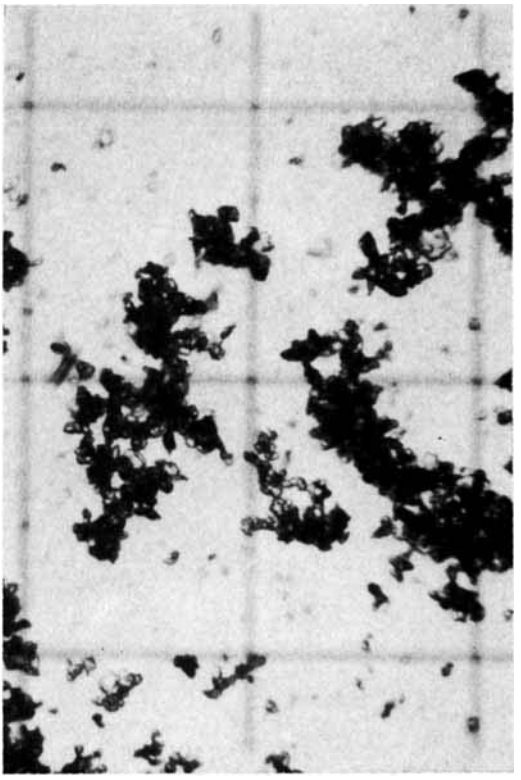

Fig. 10.—Photomicrograph of a partially flocculated suspension after the addition of the nonelectrolyte. The width of each scale division equals 96 μ.

suspension. It was assumed that "stable floc" formation was brought about by the presence of a critical amount of nonionic surfactant acting in combination with the polymer and electrolyte. In succeeding batches of the formula, the degree of overflocculation increased resulting in a suspension with poor drainage characteristics. The basic suspension was examined with the apparatus in order to determine the agent or agents actually responsible for flocculation.

A series of desorption curves were run using a $^1/_{50}$ dilution of the 4% suspension in additional suspending vehicle. Further dilutions ($^1/_2$, $^1/_4$, $^1/_8$, $^1/_{16}$, etc.) were made by replacing half of the total volume in each step with an equal volume of either distilled water or a series of different partial vehicles. The partial vehicles consisted of the complete vehicle formula minus one of the components, such as sodium chloride or polysorbate 80. The results are summarized graphically in Fig. 7.

Suspensions diluted with partial vehicles that contained sodium chloride did not deflocculate. These systems were all fully flocculated and maintained a zero ZP throughout the range of dilution. Dilution with water produced a smooth deflocculation or desorption curve after the third dilution step. Dilution of the suspension with a salt-free vehicle containing all vehicle components minus sodium chloride also produced a desorption curve. In this system, deflocculation was also initiated after the third dilution step when the salt concentration was

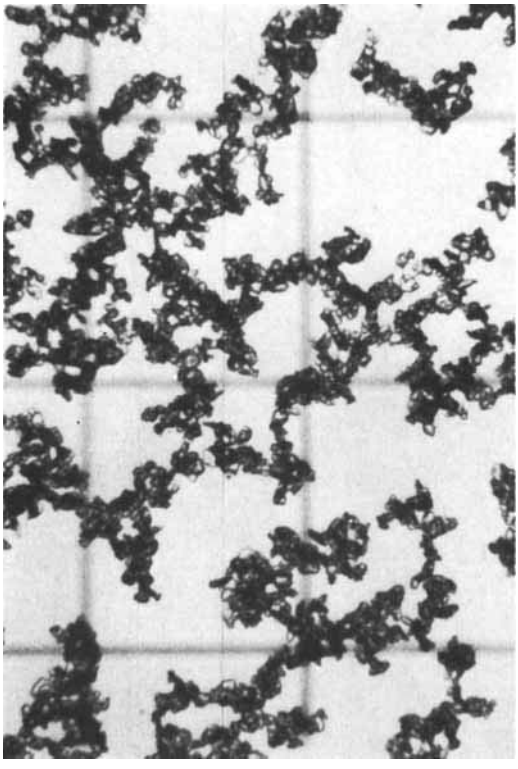

Fig. 11.—Photomicrograph of the suspension completely flocculated with the required concentration of McIlvaine buffer. The width of each scale division equals 96 μ.

reduced below 0.106%. However, the sigmoidal shape of the deflocculation curve in the region between 0.016 and 0.013% sodium chloride was apparently the result of a differential desorption.

In differential desorption, as the concentration of electrolyte and surfactant polymer is reduced, some suspensoid particles may begin to develop their own integral electrical double layer, a manifestation of which is the increase in ZP while other particles remain either partially or fully flocculated. The difference in the rate of desorption between smaller ions and larger polymer molecules apparently produces this effect.

In analyzing the desorption curves in Fig. 7, it is clear that the presence of a small concentration of electrolyte provided the necessary driving force to produce particle flocculation. However, it is not readily apparent in this study of desorption whether this is accomplished by destroying the integrity of the particles coated with adsorbed surfactant-polymer through a salting out process with electrolyte, or forcing interparticle bridging with polymer-surfactant and electrolyte. In any case, reducing the concentration of electrolyte in the formula improved drainage and the physical stability of the over-all suspension. These results also showed that an excess of salt was not required to produce "stable floc" formation.

Flocculation in the Absence of an Electrolyte.—In another example, primary flocculation was initiated in the absence of an electrolyte. Nonionic surfactants, such as polysorbate 80, acting in combination with certain other nonelectrolytes at a critical ratio of surfactant to nonelectrolyte, have the ability to produce partially or fully flocculated suspensions. However, with respect to ZP, since these systems are hydrophobic drug particles, some source of ions is required in the environment of the particle to establish a surface charge and/or electrical double layer. Therefore calculated ZP values of minus 10, 20, or 30 mv. have little or no practical significance for systems composed of neutral drug particles, nonelectrolytes, and nonionic surfactants. However, with the introduction of electrolyte, a degree of stability with respect to the ZP of the suspensoid is realized and a trend toward either deflocculation or flocculation can be ascertained.

Figure 8 shows complete adsorption and desorption curves for a drug suspension that was partially flocculated with a combination of a nonionic surfactant and a nonelectrolyte. "Stable-floc" formation was initiated with the addition of 0.03 ml. of 0.5 *M* McIlvaine buffer and completed when a total of 40 or 50 ml. of the same buffer had been added. Photomicrographs of suspensions are presented in Figs. 9–11, respectively, that show (*a*) the condition of deflocculation before the critical concentration of nonelectrolyte was added, (*b*) partial flocculation after the addition of the nonelectrolyte, and (*c*) complete flocculation when the buffer was added. The addition of electrolyte to the system in the absence of the required nonelectrolyte produces a dispersed or deflocculated system. Visual and photomicro-

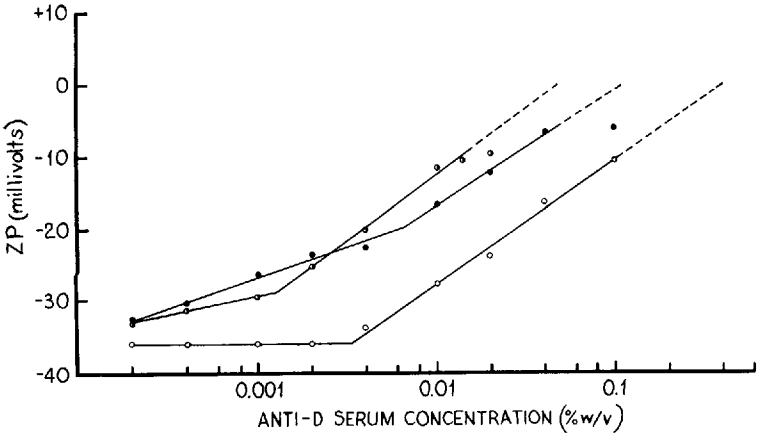

Fig. 12.—ZP titration curves for 0.01% purified silica dispersed in 0.45% NaCl and flocculated with various anti-D sera. Key: ◑, improved formula *A*; ●, control system *B*; ○, original formula *C*.

scopic inspection of these three suspensions were used to confirm the authors' findings with the apparatus.

Therefore, a physically stable suspension of the drug was developed using a calculated amount of buffer to maintain pH control and insure complete floc formation.

Flocculation with Anti-D Sera Using Purified Silica.—The final example is drawn from experiences with a biological application in determining the flocculating efficiency of several anti-D sera, available as diagnostic reagents for Rh blood typing. It was felt that a suitable product could be developed with the proper ZP for agglutination testing. Purified silica was chosen as a substitute for red blood cells (RBC's) because of the difficulties involved in working with blood dispersions (21).

Approximately 500 ml. of a 0.01% purified silica dispersion in 0.45% sodium chloride solution was prepared. The ZP of such a system (approximately −30 mv.) is comparable to the value for human blood dispersions in saline solution (22). Increments of a $^1/_{100}$ dilution of anti-D serum in 0.45% sodium chloride solution were added to each purified silica dispersion. The EM of the system was determined after each addition of reagent. After a total of 0.02% serum reagent had been added, undiluted portions of serum were used to complete each ZP titration.

The results of this study are presented in Fig. 12. The flocculating efficiency of the improved formula *A* was found to be greater than a control system *B* or the original formula *C*. Recommendations for product improvement were made on the basis of a previous study of the original formula *C*. The amount of anti-D serum required to obtain complete flocculation was based upon an extrapolation of linear portions of each test curve to the isoelectric point (zero ZP). The length of the concentration-induction period before flocculation began was also related to the efficiency of the diagnostic reagent. The ranking of the three anti-D sera according to flocculating efficiency for purified silica correlated very well with results of blood-test agglutination with human RBC's.

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Notes

Method for the Direct Measurement of Acetylsalicylic Acid in Human Blood

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A procedure was developed for directly determining acetylsalicylic acid in human blood specimens. The method instantly stops enzymatic hydrolysis, removes salicylic acid and conjugates of salicylic acid by reaction with ceric ammonium nitrate, automatically hydrolyzes acetylsalicylic acid, and determines the resulting salicylic acid fluorometrically. It sensitively measures acetylsalicylic acid in the presence of salicylic acid, salicylamide, salicyluric acid and salicylic acid ether glucuronide (*O*-carboxyphenyl glucuronide).

SEVERAL recent publications have reported acetylsalicylic acid (ASA) concentrations in the blood of humans following the ingestion of ASA-containing proprietary preparations (1–3). In each case, ASA was calculated as the difference between the level of salicylic acid (SA) in the hydrolyzed and unhydrolyzed serum (1) or in the blood extracts (2, 3). "Difference" procedures can be characterized by

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