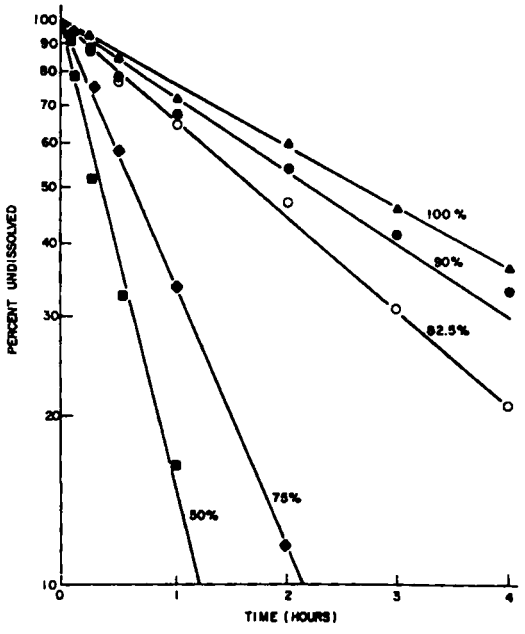

Fig. 2.—Semilog plot of per cent aspirin dissolved *vs.* time (data from Fig. 1 graph) illustrates solubility rate variations with concentration of ethylcellulose on coating.

indicates the mechanism is one of dialysis with ethylcellulose as the more impermeable film, though the literature indicates water vapor transmission through both films is similar (6). The combination of ethylcellulose and methylcellulose appears to be slowly soluble at the 50/50 level. This does not appear, however, during the length of the solubility run. The films containing the higher ratio of ethylcellulose seem to be dominated by the ethylcellulose and appear to be insoluble in the pH 4 buffer. Kanig and Goodman (6) show that this situation appears with ethylcellulose and polyvinyl pyrrolidone combinations; nevertheless, concentration changes as low as 7.5% increase in ethylcellulose and 7.5% decrease in methylcellulose (75/25 to 82.5/17.5) show a definite change in solubility rate. The influence on water vapor transmission and therefore the change in aspirin solubility seems to be significant for relatively smaller percentage changes in composition.

SUMMARY

From a purely practical pharmaceutical standpoint it is evident from the data presented that the Wurster apparatus is a valuable tool for preparing research quantities of sustained-release medications. Continuity of films and evenness of coating is possible and reproducible. The coated crystals are suitable for compression into fast disintegrating tablets that provide sustained release of aspirin. It has been shown that the solubility rate of aspirin coated with different concentrations of ethylcellulose and methylcellulose can be sustained. It has also been shown that the ratio of the two cellulose polymers affects the solubility rate, the solubility rate being inversely proportional to the concentration of ethylcellulose. This has been reflected in the solubility rate curves as shown.

REFERENCES

(1) Wurster, D. E., This Journal, **48**, 8(1959).
(2) Wood, J. H., and Syarto, J., *ibid.*, **53**, 877(1964).
(3) Brudney, N., and Toupin, P. Y., *Can. Pharm. J.*, **94**, 18(1961).
(4) Lindlof, J., WARF, personal communication.
(5) Pilot Plant Operation, Wurster Coating and Granulating Process, Wisconsin Alumni Research Foundation (WARF) Communication.
(6) Kanig, J. L., and Goodman, H., This Journal, **51** 77(1962).

Physical Stability Testing of Pharmaceuticals

By JAMES E. TINGSTAD

The importance of physical stability of pharmaceuticals and methods of testing physical stability of tablets, capsules, suspensions, emulsions, solutions, and ointments are discussed.

Physical stability is an important problem to product formulators for three primary reasons.

Appearance.—Physicians, pharmacists, patients—all expect pharmaceutical products to look fresh, elegant, and professional no matter how long they sit on the shelf. Any slight change in physical appearance—like fading of a color—may cause these people to lose confidence in that particular product.

Uniformity.—Since most products are sold as multiple-dose packages, the formulator has to make sure that the patient receives the same amount of active ingredient in each dose. A cloudy solution or a broken emulsion means that the patient is in danger of being overdosed or underdosed.

Availability.—The formulator's ethical responsibility to the patient does not end with providing a uniform dose. If the active ingredient is not absorbed, he has failed that patient just as much as if he gave him a worthless placebo. Therefore, the formulator has to make sure that the active ingredient is just as

Received August 30, 1963, from the Upjohn Co., Kalamazoo, Mich.
Accepted for publication October 7, 1963.
Presented to the Industrial Pharmacy Section, A. Ph. A., Miami Beach meeting, May 1963.

available for absorption when the product is 1 or 2 years old as it is immediately after manufacture.

Thus it is evident that physical stability is important because it can affect the therapeutic activity of a product just as much as chemical stability.

TABLETS

A physically stable tablet should retain its original size, shape, weight, and color under normal handling and storage conditions throughout its shelf life; the *in vivo* availability of its active ingredients should not change appreciably with time.

There are two tests, among others, that are helpful in determining whether a tablet will remain intact from the time of manufacture until the patient swallows it. First, a given weight of tablets is subjected to mild, uniform, and reproducible shaking or tumbling. Visual observation then reveals if the tablets chip, crack, or split under stress. All tablets that appear to be intact after this test are then weighed to determine how much material was worn away by abrasion. The Friabilator (1) is an inexpensive satisfactory instrument for this test. This apparatus consists of a plastic cylinder with a curved shelf in it. As it rotates, the tablets are subjected to mild abrasion and then, as they drop off the shelf, to sudden shock. Results obtained using this instrument are comparative, not absolute, and to get the most from the data they should be correlated with actual stress experience.

The second test is an actual shipping test, and this should be done for all types of products. In this test the tablets are packaged in their regular shipping container and shipped by rail, air, and truck across the country and back. When they return, careful examination will often reveal that the transportation industry can do strange things to tablets. It is also a good idea to put the surviving tablets back into the Friabilator to see if subtle changes in physical stability have occurred as a result of shipping.

High speed packaging machines are very rough on tablets, and they can be utilized at an early stage to determine the strength of a tablet formulation. There are also various "drop tests" that can be used to help determine the fragility of tablets, but they should be used in addition to, and not instead of, actual shipping tests. All these tests should be performed periodically as the tablet ages because the behavior of a freshly made tablet is often quite different from that of an aged one.

The color stability of a tablet can be determined by using any appropriate colorimeter or reflectometer. It is wise also to visually compare the color of a fresh tablet with that of an aged one because fading of a color is important only when it can be detected by the naked eye. Heat, sunlight, and intense artificial light can be used to accelerate chemical deterioration of a color, but this is most useful when comparing the stability of one color with another in the same formulation. It is important to remember, however, that the system at elevated temperatures is usually different from the system at room temperature, and that it is not always correct to assume that the same things will happen, in due time, at room temperature that happen at elevated temperatures.

Tablet hardness (resistance to crushing or fracture) can be tested by using any one of the available testers (2–4). Results can vary depending on which instrument is used, so one should avoid making comparisons about physical stability when different hardness testers have been used to gather data. It is important to test the hardness of aged tablets as well as freshly made ones because many times a physician will tell a patient to cut the tablet in two and take half (whether it is scored or not). If a tablet formulation hardens excessively as it ages, a patient trying to cut an 18-month-old tablet with a paring knife may have to use such force that half the tablet crumbles while the other half flies off the table and under the refrigerator. When that happens, both the patient and his physician lose confidence in that company's ability to make good products. On the other hand, a tablet that softens excessively during storage will not hold up under normal handling conditions. Uniform hardness is especially important when the product is a chewable tablet. Finally, it should be remembered that hardness and friability are not always related; an extremely hard tablet may not pass a test in the Friabilator because of excessive chipping.

Many times it is desirable to determine the stability of a tablet to moisture. To do this, the tablets are often stored in sealed bottles in a high-humidity cabinet and their physical deterioration followed. It should be pointed out that under these conditions this test is really a measure of the resistance of the container to moisture penetration. To find out how the tablets themselves will hold up while being used under humid conditions, an additional test should be conducted. Here the tablets are stored in a container in the humidity cabinet for 1 to 4 weeks, and the container is opened three or four times a day, just as it would be in the hands of the patient. The humidity should be varied in these tests to obtain a more complete picture of the tablet's stability to moisture.

When the formulator is satisfied that a tablet formulation will reach the patient intact and elegant looking, there is still one more vitally important test to be performed before he can consider his job finished. He has to make sure that the drug will be well absorbed by the patient throughout its shelf life. This is important enough to warrant a brief theoretical discussion.

When a systemic acting drug is tested in the clinic, the proper therapeutic dose that is determined is a function of the amount of drug given and the percentage of the dose that is absorbed. To market products that are therapeutically efficient, effective, and uniform, the formulator has to make sure that the drug is well absorbed initially and that its absorption characteristics do not change appreciably throughout the product's shelf life. Changes in absorption characteristics can be caused by polymorphism, changes in particle size, changes in the dissolution rate of the active ingredient out of the tablet, and other factors.

Levy and Nelson (5) have pointed out that the nature of the formulation does affect the absorption characteristics of drugs, and evidence has been gathered at these laboratories showing that the absorption of a drug can vary considerably depending on the age of the formulation. For example, Fig. 1 shows how blood levels obtained after oral

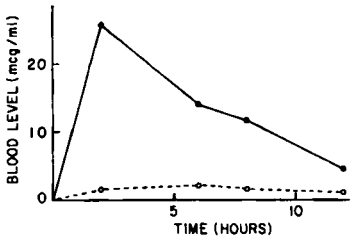

Fig. 1.—Plot showing the decrease in blood levels when an aged suspension (broken line) was administered orally to humans rather than a fresh suspension (solid line).

administration of a formulation decreased greatly as that formulation aged. Other examples could be cited, but the point to remember is this: it is unwise and dangerous to assume that just any formulation will give good drug absorption, and it is equally unwise and dangerous to assume that a well absorbed formulation will remain so throughout its shelf life.

Obviously, it is not practical to test clinically every product at regular time intervals during storage. However, there are several *in vitro* tests that can be done to help detect when changes in absorption characteristics are likely to occur. For tablets, one of the better *in vitro* tests is to determine the active ingredient's dissolution rate in artificial gastric and/or intestinal juice at 37°. The standard U.S.P. tablet disintegration apparatus can be used for these determinations (preferably without plastic disks) and ultraviolet spectroscopy can usually be used to develop a rapid assay method.

It should be emphasized at this time that dissolution rate (the rate at which the active ingredient goes into solution) is quite different from disintegration time (the time it takes for a tablet to disintegrate and the particles to pass through a standard screen). Tablets having sand as the "active" ingredient can be made so that they disintegrate very rapidly; yet blood levels of silicon dioxide would be hard to find after administering such a tablet. It has been shown (6) that disintegration time is not necessarily a good measure of dissolution rate, and it is the dissolution rate of the active ingredient that often determines the rate of absorption of that drug (5). These dissolution rate tests should be run periodically, and if no other physical changes (polymorphism, etc.) take place, an unchanging dissolution rate usually means constant *in vivo* availability. The value of these dissolution rates can be greatly enhanced if they can be correlated with *in vivo* work.

Disintegration times can be determined periodically to detect gross changes in the physical characteristics of a tablet, but this test is not sufficient unless there is a demonstrated correlation between disintegration time and dissolution rate for that particular formulation. When there is no such correlation, the dissolution rates should be determined. In the case of delayed (sustained) release formulations, release patterns are even more important and should be determined periodically during storage.

When the product is a coated tablet, the physical stability of the coat should also be investigated. Many of the above tests for plain tablets can be applied to coated tablets also.

There are two additional points concerning the physical stability testing of tablets that the formulator should keep in mind. First, it is important to recognize that elevated temperatures do not always give a true picture of what will happen, in due time, to a tablet at room temperature. For example, there are tablet formulations that swell and split at elevated temperatures; yet they remain intact indefinitely at room temperature. Also, most tablet coats will melt or soften at elevated temperatures; this seldom happens at room temperature.

Second, it is important to subject tablet formulations, especially coated ones, to freezing temperatures for at least 1 week or 2. Several times this test has revealed tablet coats that were susceptible to cracking when frozen. Subsequent shipping tests to Canada and the northern United States in the winter confirmed these observations.

GELATIN CAPSULES

It is unnecessary to spend a great deal of time discussing gelatin capsules. The capsule formulator should ascertain that: (*a*) the shells of the capsules do not soften (and stick together) or harden (and crack under slight pressure) excessively during storage, (*b*) the capsules remain intact under normal handling and shipping stress, (*c*) the color of the capsules does not change appreciably during storage, (*d*) the capsule contents, either as a powder or an oil, do not leak out of the capsule, and (*e*) the *in vitro* dissolution rate of the active ingredients does not change appreciably with time. It is especially important to test the dissolution rate of capsules at 37° rather than room temperature because gelatin capsules that dissolve in 15–30 minutes at room temperature will often dissolve in less than 2 minutes at 37°.

SUSPENSIONS

A physically stable suspension is one that can be homogeneously redispersed with moderate shaking and easily poured at any time during its shelf life, with neither the particle size distribution, the crystal form, nor the physiological availability of the suspended active ingredient changing appreciably with time.

There is not enough time to discuss thoroughly the basic physical system present in suspensions; but because of the many prevailing misconceptions about suspension systems, it is necessary to mention some of their more important theoretical aspects. Most good stable pharmaceutical suspensions are flocculated; that is, the suspended particles are physically bonded together in such a way that they form a loose, semirigid structure. This structure, in a sense, is independent of the suspension vehicle; that is, the particles hold each other up and exert no significant force on the liquid.

As a crude illustration, think of a piece of wire screen immersed in a liquid. The individual strands of wire are not supported by the liquid; rather, each strand is held in place by its neighbor. Consequently, it is the structure of the screen, and not the nature of the liquid, that determines where each strand will be in the system. Also, the wire exerts no pressure on the liquid. In the same way the particles of a flocculated suspension hold each other up, and their position in the suspension depends

primarily on the nature of their bonds with their neighbors and not upon the inherent qualities of the vehicle.

When a flocculated suspension is first made, it is well stirred and the particles are floating randomly around in the vehicle. As soon as the suspension is poured into a bottle and set on the shelf, the particles start to settle and come together. Soon (minutes to hours, depending on the system) many of the particle-particle bonds have formed and the particles continue to settle, not as individuals but as clusters of particles (agglomerates). Soon (days to weeks, depending on the system) the total structure is formed, and settling continues only because the particles are rearranging and moving closer together by forming bonds with more of their neighbors, causing the structure to compact.

Eventually, for all practical purposes, the structure becomes stable and settling stops. The sediment remains stationary like a fragile piece of wire screen sitting in the vehicle. How much volume this sediment occupies depends on the system, but in a good flocculated suspension it is usually more than half the total suspension volume, and many times it occupies all of the suspension volume. This sediment can be easily redispersed at any time by moderate shaking, and the system will then once again be completely homogeneous.

In nonflocculated suspensions, on the other hand, the particles remain as individuals, essentially unaffected by their neighbors and affected only by the suspension vehicle. These particles, being smaller and lighter than the agglomerates in a flocculated suspension, settle much more slowly. But once they have settled, they often form a rock-hard sediment on the bottom of the bottle that is extremely difficult to redisperse. These suspensions would be acceptable if one could decrease the particle size enough and increase the density and viscosity of the vehicle enough so that the particles would settle very little during the product's shelf life, but this is very hard to do in most cases. Consequently, flocculated suspensions are usually the most pharmaceutically acceptable; and, by design or by accident, most of the presently marketed suspensions are flocculated.

This discussion of suspension systems was necessary to show why some physical stability tests are valid to use while others are not. For example, Stokes law can seldom be used to examine suspension stability because it applies only to nonflocculated suspensions. And, contrary to popular belief, the many conventional viscometers are practically useless for studying physical stability of flocculated suspensions because they employ shear rates that tear the fragile structure apart, and the system thus studied has little or no relation to the actual system as it sits on the shelf. Even centrifugation (to accelerate settling) is of little value because the structure is so fragile that it breaks down under the increased force placed upon it by the centrifuge.

This can best be illustrated by imagining that a formulator has a big cardboard box full of eggs packed in a certain manner and that he wants to determine the condition of the eggs and how they are packed. To do this, he takes an axe and proceeds to open the box in a violent but effective manner. He then observes that the eggs are in

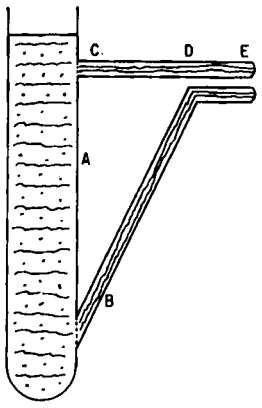

Fig. 2.—Diagram of a differential manometer. See *text* for explanation of instrument.

very poor condition and that they are packed in a very irregular and haphazard way. Poor technique leading to meaningless and erroneous conclusions? Yes, but to a certain extent this is precisely what is done to these fragile sediment structures when they are studied in the conventional manner using conventional viscometers.

How, then, should they be studied? First of all, it is necessary to determine whether the suspension under study is actually flocculated. This can be done very easily by taking advantage of the fact that the particles in a flocculated suspension support each other and exert little or no pressure on the liquid. In the apparatus shown in Fig. 2 (called a differential manometer) (7) the suspension is placed in container A and the vehicle (but not the suspended particles) is allowed to enter sidearms B and C. At point D the two sidearms are parallel and are at the same height (B appears to be lower than C only because the drawing is two-dimensional). If the suspension is flocculated, the liquid in the two sidearms will travel the same distance (*e.g.*, to point E) because of equal hydrostatic pressure (B and C are inclined slightly after point D to magnify slight differences in pressure). If the suspension is not flocculated, the weight of the suspended particles pressing down on the liquid will be greater at the bottom of container A than at the top; consequently, the hydrostatic pressure will be greater in B, and the liquid will travel further in that sidearm.

Once it has been determined that a suspension is flocculated, two simple experiments can be set up to study its physical stability. First, a measured volume of the formulation is stored in a standard size graduated cylinder and the height of the sedimented solids measured periodically by visual observation. This test does not disturb the system at all, and it is very useful for determining stability. For example, if the sediment settles rapidly to a height which is 90% of the total suspension height and then settles no further, the formulation is probably satisfactory. This technique is also useful in determining which is the best of a number of formulations. All other things being equal, the suspension with the largest sediment volume is the best formulation.

While the above test is an excellent one for observing gross characteristics of sediments, it is also desirable to be able to look inside these structures and observe the settling behavior (or rearrangement) of the agglomerates. Fortunately, there is an excellent piece of equipment available to do just

that—the Helipath attachment used with a conventional Brookfield viscometer.

This instrument consists of a slowly rotating T-bar spindle which descends slowly into the suspension as it rotates. The dial reading on the viscometer is a measure of the resistance that the spindle meets from agglomerates present at various levels in the sediment. A higher dial reading (greater apparent viscosity) means that more particles are present at that particular level in the sediment. Repeating the test at various time intervals, each time using a fresh undisturbed sample, gives an excellent picture of how the particles are rearranging (settling) within the sediment structure as the preparation sits on the shelf. It is necessary to handle these samples carefully so that the structure is not disturbed while removing the sample from the shelf. The T-bar spindle disturbs the structure slightly as it rotates, but since it descends as it turns, it continually encounters new, essentially undisturbed material.

This instrument is also very useful in comparing the stability of various formulations of the same product. Figure 3 shows the Helipath profile of two slightly different suspensions which were made at the same time. It can be seen that the agglomerates in Preparation I are settling faster than those in Preparation II (the sedimentation volume of Preparation II was greater). Preparation II proved to be the better suspension. Thus, results obtained using the Helipath, coupled with observations made on sediment volume, can reveal a great deal of useful information about suspension stability.

The important point to be remembered here is that by using the Helipath one can study the state of the sediment without first shaking the suspension to make it homogeneous. In most other viscometric tests the suspension is shaken vigorously to insure

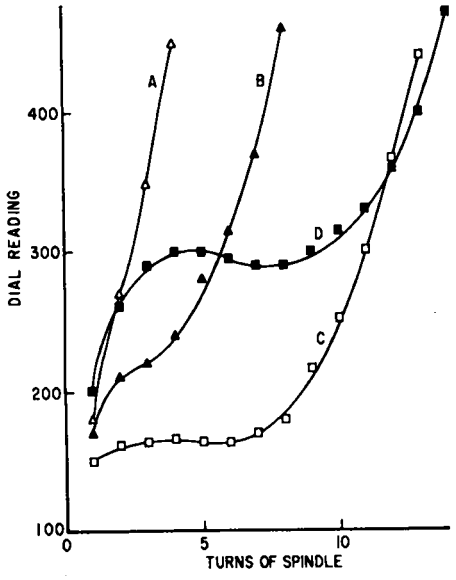

Fig. 3.—Plot illustrating data obtained using Helipath attachment to a conventional Brookfield viscometer. Curve B is for Suspension I (aged 6 months), curve A is for Suspension I (aged 9 months), curve D is for Suspension II (aged 6 months), and curve C is for Suspension II (aged 9 months).

homogeneity, then the preparation is poured into the viscometer. This, in a sense, is closely akin to opening egg cartons with axes.

These two tests are by no means the final word in suspension stability testing. There is a great deal of work that can and should be done to design still better tests. For example, it would be very helpful if the aging of a suspension could be accelerated in a way that corresponds to the actual aging that takes place on the shelf. This is being investigated in these laboratories, and preliminary results are very encouraging.

As mentioned in the definition of a stable suspension, it is also important to detect changes in particle size distribution, crystal form and, when necessary, the physiological availability of the active ingredient. The particle size distribution of the suspended drug is important because it may affect the appearance and taste of the preparation and the *in vivo* absorption of the active ingredient (*e.g.*, griseofulvin and some sulfonamides). The Coulter counter, an electronic particle counter and sizer, is an excellent tool for determining particle size distribution for particles greater than 0.5 μ. Of course, the microscope can also be used to estimate particle size distribution, but this is a tedious procedure. Changes in crystal form can be detected using X-ray diffraction or microscopic examination. If tests indicate that significant changes are taking place in crystal form, etc., it is imperative that further clinical work be done on aged formulations. The truth of this statement is clearly illustrated in Fig. 1, where blood levels dropped to practically zero when a slightly aged formulation was administered rather than a fresh one.

The following additional observations may be helpful to the suspension formulator:

Subtle chemical degradation of an ingredient can affect the physical appearance of a suspension. Recently, a sterile suspension containing a steroid ester was formulated in these laboratories. The physical appearance of the formulation, excellent at first, broke down completely within 1 month. Chemical assay for total steroid indicated good chemical stability, and considerable time and effort were spent trying to determine the cause of the trouble. Eventually, an assay was developed that differentiated between the steroid ester and its hydrolysis product, the free alcohol. It was then found that the ester was rapidly hydrolyzing to the free alcohol and that the suspending system which worked well for the ester was totally inadequate for suspending the alcohol.

Changes in crystal form can also adversely affect a suspension, either in its physical appearance or in its *in vivo* absorption. Suspensions of amorphous novobiocin acid (well absorbed) become useless in a short period of time because the drug reverts to the crystalline acid which is poorly absorbed.

It is wise to subject all suspensions to cycling temperatures; that is, to store them at elevated temperatures and then in a refrigerator, repeating this procedure several times. At elevated temperatures more of the drug will go into solution; then when the preparation is stored in the refrigerator, the excess drug in solution reprecipitates. In this way the tendency of the drug particles to grow in size can be studied.

Shipping tests should always be performed on

suspensions. In the past, several problems have been averted by observing that certain apparently good suspensions broke down during shipping. Also, the suspension should be frozen to make sure that shipping the suspension at freezing temperatures will not adversely affect it.

The pouring qualities of suspensions at refrigerated temperatures should be observed. Many patients store their fluid medications in the refrigerator, and it would prove embarrassing to market a suspension before it was discovered that it will not pour after being stored in a refrigerator.

Microscopic examination of suspension systems often gives a good picture of the state of the sediment (*e.g.*, whether the particles are interacting with each other).

Finally, when recent production lots of an established suspension product suddenly break down physically, it often helps to take a careful look at all of the ingredients to make sure that no supplier changed his manufacturing process. In the past, several such difficulties have been resolved by determining that a supplier changed his manufacturing procedure without notifying anyone. pH is another factor that should be suspected whenever things suddenly go wrong in suspension manufacture because it can have a profound effect on suspension stability.

EMULSIONS

A physically stable emulsion is one that can be homogeneously redispersed to its original state with moderate shaking and can be easily poured at any time during its shelf life.

Emulsion systems are just as complicated as suspension systems, if not more so. Again, time does not permit a thorough discussion of emulsion systems, but it is necessary to mention some of their more important theoretical aspects because the tests chosen to study physical stability should be based on certain assumptions concerning the basic physical system involved. Since most of the important pharmaceutical emulsions are oil-in-water, the discussion will be confined to this type. However, many of the remarks and tests apply to either oil-in-water or water-in-oil emulsions.

There is a good chance that most stable emulsions, especially those with a high oil content, are flocculated in much the same way as stable suspensions and that the stability of most emulsions depends on the strength of the interfacial film surrounding the dispersed oil particles. If these assumptions are correct, rate of coalescence of the internal phase is a valid measure of emulsion stability but, contrary to popular opinion, the rate of creaming is not. In these laboratories, several stable emulsions have been encountered that have a high initial creaming rate. Of course, it is best for the sake of appearance if the volume of the cream occupies as much of the emulsion volume as possible. This situation is similar to that encountered in suspensions.

When formulating an emulsion, it is important to be able to select the most stable formula, and there are two simple tests that can be used to do this. First, the various formulations are subjected to heat (50–70°) and the gross stability of the emulsions determined by visual observation or by turbidimetric measurement. The emulsion that is most stable to heat is usually the most stable at room temperature. However, this is not always the case because the systems at 60° are not the same as those at room temperature. The solubility of the emulsifiers in the two phases will be different, and the change in solubility with temperature may not be the same for both phases. Furthermore, the high temperature may chemically degrade some of the ingredients so that a distorted picture of physical stability is obtained.

The second method for estimating emulsion stability is often called the "coalescence time" test (8). In this test the emulsion system is prepared in two parts, one containing all the hydrophilic materials dissolved in water and the other containing all the hydrophobic materials dissolved in oil. Any materials that are insoluble in both phases should be dispersed in the phase that is most compatible with that material. The water phase is placed in a container, and the oil phase is carefully layered over it. Then a drop of the oil phase is introduced (*e.g.*, through a syringe attached to the bottom of the container) at the bottom of the aqueous phase; it rises through that phase and comes to rest at the interface between the two layers. After a certain length of time, the oil droplet will coalesce with the oil layer. The longer the drop takes to coalesce, the more stable the emulsion should be. This test is only roughly quantitative, but many times it can be very useful for detecting gross differences in emulsion stability at room temperature.

It was stated earlier that particle size distribution is the most valid criterion to use in determining emulsion stability. The Coulter counter is an excellent instrument for determining the particle size distribution of oil droplets larger than 0.5 μ. A good optical microscope is another useful tool, but this involves tedious work. Knoechel and Wurster (9) have refined the microscopic technique to good advantage. The Helipath attachment to the Brookfield viscometer is also useful in emulsion stability work. It gives a rapid semiquantitative picture of how the droplets (or agglomerates) are rearranging (rising) in the system.

Three additional observations on emulsion stability work may be of interest to the formulator. First, it should be recognized that temperature (low as well as high) can have a drastic effect on the behavior of emulsions. Several years ago in these laboratories an emulsion was formulated that was stable at room temperature but broke down when it was stored in a refrigerator. The reason for this was that one of the oil soluble emulsifiers precipitated out at refrigerator temperatures. This disturbed the system enough so that the emulsion broke in a few days.

Second, in preparing lots of an emulsion formulation for stability testing, it is best if at least two lots are prepared using production homogenizing equipment. Different kinds of homogenizers often produce different results, and even different sizes of the same kind of homogenizer can produce emulsions with different stability characteristics. Thus, stability results obtained solely from bench size lots may not be applicable to the finished product made in production.

Finally, following the stability of the emulsion vehicle without the oil phase being present (this can be done with suspensions also) will often yield

additional useful information about stability of the product.

SOLUTIONS

A physically stable solution retains its original clarity, color, and odor throughout its shelf life.

Solutions are relatively simple systems; the main concern of the formulator is that they retain their clarity. One should never depend on rough visual observation under ordinary light to determine clarity, especially in the case of a parenteral product; many hazy solutions pass this type of inspection. A better idea of a solution's clarity can be obtained by shining a microscope light through a diaphragm (to concentrate the beam) into the solution. Undissolved particles will scatter the light, and the solution will appear hazy. The Coulter counter can also be used to determine solution clarity, but light-scattering instruments are the most sensitive means of establishing that a solution is perfectly clear. Ordinarily, however, it is not necessary to use these more expensive instruments in this type of stability work.

It is important that a solution remain clear over a relatively wide temperature range (4–47°). At the lower temperatures one of the ingredients may precipitate because its solubility at that temperature is too low. At the higher temperatures homogeneity may be destroyed by particles flaking off glass containers or rubber closures or by chemical degradation of one of the ingredients. Further information about stability can be obtained by cycling the preparation between 4° and 47°. There are some systems that will yield a precipitate only after several weeks of this treatment, so a single 2 or 3-day cycling experiment is not always sufficient. Changes in pH will often cause precipitation of ingredients in solution, especially when these ingredients are soluble salts of insoluble acids or bases. Therefore, pH should always be followed in studying the physical stability of solutions.

When the solution is a sterile preparation, the formulator should keep in mind that foreign substances in clarifying and sterilizing filters (metals, etc.) are at times capable of causing precipitation reactions that would not ordinarily take place. Also, the filters may adsorb an ingredient and thus change the composition of the preparation. For stability work it is important to use the same type of filter that will be used in production.

OINTMENTS

A physically stable ointment retains its homogeneity and its consistency throughout its shelf life.

Hydrophobic ointments (*e.g.*, petrolatum) may be thought of as high-viscosity suspensions of active ingredients in a nonreacting vehicle. Thus, changes in particle size distribution, crystal form, etc., of the active ingredient are seldom a problem. The main physical stability problems encountered with ointments are bleeding and changes in consistency (due to aging or to changes in temperature). Bleeding occurs when fluid constituents (*e.g.*, mineral oil) separate at the top of the preparation. Visual observation is usually sufficient to detect this, but there is no known way to accelerate this process so that tendency to bleed can be predicted.

It is important to be able to define in a quantitative way the consistency of ointments because an ointment that is too soft is messy to use, and one that is too stiff is difficult to extrude out of a tube and apply. One of the instruments that is presently used to determine ointment consistency is the Penetrometer, an apparatus that allows a pointed weight to penetrate the ointment under a measurable force. The depth of penetration is a measure of the consistency of the ointment. The Helipath attachment used with a high-viscosity viscometer can also be used for this type of study.

At present the performance of another instrument, the Burrell Severs rheometer, is being investigated in these laboratories. In this instrument the ointment is loaded into a cylinder and a measured force (compressed air) extrudes a certain amount of ointment onto a receiver. The amount of ointment extruded is a measure of the consistency of the ointment. The manufacturer supplies a loading device which sucks the ointment into the cylinder, but this creates air pockets within the ointment and leads to erratic results. Much better success has been obtained in loading by melting the ointment, pouring it into the cylinder and allowing the system to cool and come to equilibrium at constant temperature. It should be recognized that remelting an ointment in this way might cause irreversible changes in the system, so it is best to obtain a sample of the ointment while it is still fluid during the original manufacturing process.

These experiments have revealed two interesting facts about ointments that should be of interest to the formulator.

First, ointments have a considerable amount of structure, and this structure takes at least 48 hours to form after the ointment has been melted. It has been found that the consistency of an ointment that has been standing 72 hours after melting is considerably more solid than that of the same ointment that has been standing for only 24 hours. It is evident from these results that rheological experiments performed on freshly made ointments may yield erroneous data. In any case, it should be determined that the structure of the ointment has reached equilibrium before any tests are performed.

Slight changes in temperature (1 or 2°) can greatly affect the consistency of an ointment. The "apparent" viscosity of one ointment formulation was twice as high at 23° as it was at 25°. Therefore, it is essential that all rheological tests on ointments be done at constant and controlled temperatures.

The physical stability problems encountered with hydrophilic creams (o/w emulsions) are similar to those of ointments and to problems discussed under *Emulsions*.

CONCLUSIONS

There are seven "rules to live by" which, if kept in mind when studying the physical stability of any system, will help the formulator to market the best possible products.

(*a*) Often the most valuable instrument in the formulator's possession is an established product whose system is similar to the one being studied. Most good physical stability tests are comparative, and using an established product as a control will greatly increase the accuracy of stability predictions.

(*b*) It is best to know as much as possible about

the basic physical systems involved in the formulations under study. A few shrewd observations early in the game will often save a great deal of trouble and effort later on.

(*c*) When using heat to accelerate a physical aging process in a heterogeneous preparation, it should be remembered that the system at elevated temperatures is different from the system at room temperature; consequently, extrapolation to room temperature stability must be done with care and caution.

(*d*) The analytical method employed to study disperse systems (suspensions, etc.) must not be too harsh because the disturbed system may have little or no relationship to the system as it sits on the shelf.

(*e*) A change in pH can often have a profound effect on physical stability; consequently, all physical stability data should include periodic measurement of pH whenever possible.

(*f*) One of the most important aspects of physical stability—physiological availability of the active ingredient—is often the most neglected. All good stability studies should include *in vitro* tests (dissolution rate, etc.) that will help detect when changes in *in vivo* absorption characteristics are likely to occur.

(*g*) Before final approval is given to the physical stability of any product, it should be taken home and used just as a patient would use it (without actually taking the medication). This "home trial" often reveals physical stability problems that never arise in the laboratory.

REFERENCES

(1) Shafer, E. G. E., Wollish, E. G., and Engel, C. E., THIS JOURNAL, **45**, 114(1956).
(2) Endicott, C. J., Lowenthal, W., and Gross, H. M., *ibid.*, **50**, 343(1961).
(3) Fairchild, H. J., and Michel, F., *ibid.*, **50**, 966(1961).
(4) McCallum, A., Buchter, J., and Albrecht, R., *ibid.*, **44**, 83(1955).
(5) Levy, G., and Nelson, E., *J. Am. Med. Assoc.*, **177**, 689 (1961).
(6) Schroeter, L. C., Tingstad, J. E., Knoechel, E. L., and Wagner, J. G., THIS JOURNAL, **51**, 865(1962).
(7) Kelly, W. J., *Ind. Eng. Chem.*, **16**, 928(1924).
(8) Cockbain, E. G., and McRoberts, T. S., *J. Colloid Sci.*, **8**, 440(1953).
(9) Knoechel, E. L., and Wurster, D. E., THIS JOURNAL, **48**, 1(1959).

Notes

Assignment of the *N*-Methyl Hydrogen NMR Peaks of Caffeine

By THOMAS G. ALEXANDER and MILLARD MAIENTHAL

The NMR spectrum of caffeine exhibits a separate peak for each of the three *N*-methyl groups. Assignment of each peak to a specific *N*-methyl group was made by comparison of the spectrum of caffeine with those of two caffeine homologs, in each of which one of the *N*-methyl groups is replaced with an *N*-ethyl group.

IN THE nuclear magnetic resonance spectrum of caffeine published by Bhacca, *et al.* (1), *N*-methyl hydrogen peaks are shown at 6.60, 6.41, and 5.99 p.p.m. However, there is no specific assignment of these to the 1, 3, and 7 positions. We undertook this study to make such assignments.

The *N*-methyl hydrogen peaks of caffeine can be selectively removed from the spectrum by (*a*) the substitution of the methyl hydrogen atoms with deuterium atoms, (*b*) the substitution of methyl groups with ethyl groups, or (*c*) the substitution of methyl groups with hydrogen atoms. The last technique might result in a significant difference in the chemical shift of the remaining *N*-methyl hydrogen peaks from the corresponding ones of caffeine, since a hydrogen atom would have considerably less inductive effect upon the rings than a methyl group. Because it was more convenient to synthesize two of caffeine's higher homologs than to prepare the partially deuterated caffeines, the second approach was used. The homologs were synthesized by treating theobromine and theophylline with ethylating agents. Samples of these compounds and of caffeine were dissolved in deuterated chloroform and their NMR spectra were obtained (Fig. 1).[1] Tetramethylsilane was used as an internal reference standard.

Caffeine—(1,3,7-Trimethylxanthine).—Eastman's white label was used.

Ethyltheobromine—(3,7-Dimethyl-1-ethylxanthine).—The sample was prepared by the method of Rodionov (2). When recrystallized from water, the product melted at 161–163°.

Ethyltheophylline—(1,3-Dimethyl-7-ethylxanthine).—The sample was prepared by a modification of Schmidt's method (3). One gram of theophylline and 2 ml. of ethyl sulfate were refluxed in 20 ml. of 5% NaOH for several hours. The solution was kept basic during the refluxing by the addition of 5% NaOH. The product, when extracted with chloroform and recrystallized from water, melted at 150–151°. (Schmidt reported a value of 154°.)

As anticipated, the spectra of each of the caffeine homologs consisted of two sharp *N*-methyl peaks, a triplet, a quartet, and a single olefinic hydrogen

Received August 20, 1963, from the Division of Pharmaceutical Chemistry, Bureau of Biological and Physical Sciences, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.
Accepted for publication December 2, 1963.

[1] A model A-60 Varian NMR spectrometer was used in these studies.