CHAPTER 1

Introduction and Technique

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1.1 INTRODUCTION

One of the "beneficial" concoctions, in the year 1720, for the treatment of patients with tuberculosis was prepared in the following way:[1]

> Take of new Milk two Gallons, distil it with Mint, Roman Wormwood, of each two Handfuls, to a Gallon. Then Take of Garden Snails, washed first in common Water, and then Small Beer, half a Peck, of Earth-worms slit and wash'd a Pint, of Angelica a Handful and half, Agrimony, Betony, Rue, of each a Handful. Put the Herbs in the bottom; upon these the snails and Earthworms, and upon the top of all lay the Shavings of Hartshorn half a Pound, of Cloves an Ounce, of Saffron three Drams, Infuse them in two Quarts of Syder, and a Quart of the best Malaga Sack, and then distil them in an ordinary Still. These Liquors must be drank plentifully.

The reader's thoughts may tumble at once to the opening scene of *MacBeth* or may be betrayed by a fleeting smile on the lips or in the eyes, but such or similar preparations were accepted and used, not only in the eighteenth century, but also well into modern times. It is doubtful that the above concoction could be produced repeatedly, and it is unlikely that any pharmaceutical analyst could write more than the vaguest of specifications for the final product. This is in sharp contrast to the specifications for the conquerors of tuberculosis: isoniazid, *p*-aminosalicylic acid, and streptomycin. Not only do these substances possess specific characteristics, but their production is controlled by exacting specifications that guarantee a uniform and therapeutically acceptable product. This, then, is the home country of the pharmaceutical analyst; the limit tests, the titrations, the precipitations, and many other procedures form the base for this discipline and guarantee progress in the field of the pharmaceutical sciences.

Definitions tend to be confining, and it is with some reluctance that this discipline is defined as the subject which deals with the quantitative analysis of those chemicals and dosage forms associated with the practice of pharmacy. Some authors prefer to widen the definition to include the determination of drugs or their derivatives in plants and biological fluids. Regardless of the definition, the subject is usually taught as a separate entity in most pharmacy schools. Some colleges offer a number of courses in pharmaceutical analysis, and it is possible, therefore, for the student to specialize and eventually to embark on a career in this area of pharmacy. It almost seems redundant to say that a study of the techniques inherent in the analysis of pharmaceuticals serves at least two other purposes. First, the foundation of science is analytical chemistry. Therefore, there is a direct relationship between pharmaceutical analysis and the pharmaceutical sciences in general, and the techniques learned here may be readily transcribed to other parts of the pharmacy curriculum. Measurements are particularly important in research, and it is almost mandatory that all students destined for graduate school receive some

training in the analytical techniques common to pharmacy. It is regrettable, however, that many potential pharmacists approach this discipline with misgiving, forgetting that pharmacy itself is grounded in the production and dispensing of a quality product, which can be obtained only by a meticulous approach to detail. Second, therefore, the analysis of pharmaceuticals provides a training ground for the accuracy expected of the pharmacy graduate. It is here that the student will learn the importance of the fourth decimal in the weighing process and the significance of the limits placed on the quantity of drug in any particular dosage form. The student is advised to read Ref. 2 (at the end of the chapter) for a more detailed discussion of pharmaceutical analysis and dispensing accuracy.

One of the inherent dangers in a course in pharmaceutical analysis is that the student deals with isolated techniques which, in actual practice, are combined, to one extent or another, to provide total control of the quality of the dosage form. For example, a typical laboratory exercise might demand that the student carry out an analysis of tolbutamide. This determination can be carried out by dissolving the substance in alcohol and titrating the resulting solution with standardized sodium hydroxide solution. The student gains the impression that this represents a total analysis of the product. Much has been written on this subject, and it is beyond the scope of this book to discuss the concept of total quality control of pharmaceuticals. However, to help the student orient his thinking, Table 1.1 presents the quality-control profile of a drug that is commonly handled by the pharmacist. The table is self-explanatory, but, as an exercise, the student might determine the actual number of analytical operations that must be performed during the production of this particular drug. It is obvious that the final assay represents but one of the many analytical manipulations carried out in this entire process. The table also illustrates the importance of the quality-control laboratory in the production of pharmaceuticals. Most industrial processes continue only on clearance by the person in charge of the quality-control section. The importance of this man in the industrial complex is often obscured by other factors, but his position in the company often gives him a maximum of authority over production and distribution. His decisions are, in most instances, subject to review by only the most senior management officials of the company.

The pharmacist's concern with quality is a historical fact. At times, however, one feels that the modern professional has lost or is only paying lip service to this basic concept of drug quality. To be fair, most senior pharmaceutical organizations and the ethical industry take a keen interest in and often participate actively in the preparation of the standards laid down in such compendia as the *United States Pharmacopeia*, the *National Formulary*, and the *International Pharmacopoeia*. The *British Pharmacopoeia* deserves special mention because it has diligently served an empire, and the world, over countless generations. These and other pharmacopeias form the

TABLE 1.1: The Quality-Control Profile of a Drug (A Case History Illustrating the Quality-Control Steps Required by the USP XVI for Penicillin G Tablets USP*)

Active ingredient	Tests required†		Official compendia
Penicillin G potassium or sodium	Solubility	Penicillin G content	FDA,‡ USP
	Appearance	Total assay	
	Color	Heat stability	
	pH	Clarity	
	Toxicity (safety)	Odor	
	Loss on drying	Crystallinity	
	Potency		
Reagents			
Potassium hydroxide	Appearance	Solubility	USP
	Color	Heavy metals	
	Odor	Insoluble substances	
	Identity	Assay	
Buffers			
Sodium phosphate monobasic	Appearance	Iron	USP
	Assay	Nitrogen compounds	
	Solubility	Sulfate	
	Loss on drying	Heavy metals	
	Chlorides		
Excipients			
Magnesium stearate	Appearance	Identity	USP
	Color	Arsenic	
	Odor	Lead	
	Solubility	Assay	
	Loss on drying		
Corn starch	Appearance	Loss on drying	USP
	Color	Identity	
	Odor	Microscopic examination	
	Solubility	Residue on ignition	
	Reaction	Iron	
Milk sugar	Appearance	Taste	USP
	Color	Residue on ignition	
	Odor	Specific rotation	
	Clarity of solution	Other sugars	
	Solubility	Heavy metals	
	Identity		
Sodium benzoate	Loss on drying	Identity	USP
	Appearance	Alkalinity	
	Color	Heavy metals	
	Odor	Chlorinated compounds	
	Solubility	Assay	
Precipitated chalk	Appearance	Loss on drying	USP
	Color	Acid-insoluble substances	
	Odor	Barium	
	Taste	Heavy metals	
	Solubility	Magnesium and alkali salts	
	Identity	Assay	
Finished Product			
Penicillin G Tablets USP (potassium or sodium)	Disintegration time		USP
	Weight variation		
	Loss on drying		
	Assay		

* This table is reproduced through the courtesy of *Bio-Medical Purview* and P. S. Cowles, Director of Quality Control, E. R. Squibb & Sons, New Brunswick, N.J. See Ref. 3.

† These tests form the skeleton for a host of tests run by individual pharmaceutical companies. Some manufacturers will only run the official tests, but the majority of producers have a roster of their own tests imposed on those required by official compendia and/or the Food and Drug Administration. In addition, there are many raw materials which enter into the penicillin fermentation process for which specification must be established and maintained by each manufacturer. Moreover, a variety of other excipients are used in the finished product to suit the specific manufacturing requirements of each firm.

‡ Required by *Antibiotic Monograph* issued by the Food and Drug Administration for certifiable antibiotics.

chapters of the pharmaceutical analyst's "bible," and a study of the techniques inherent within their many pages is the subject matter of this book.

There are many sources of information on drug standards, but those in the pharmacopeias are, in most instances, directly related to the legislative wishes of many world governments. These pharmacopeias form the basis of what is considered good therapeutic usage (a fact often forgotten in this age of highly advertised drugs) and also provide standards for a selected group of drugs. In addition, many tests and procedures described in such books can be readily used to ascertain the quality of nonofficial preparations. Legislatures recognize this, and it is thus possible to conclude that no field of analysis is as closely allied to law as the field of pharmaceutical analysis. This legislative concern with drug quality is understandable. The dosage forms the pharmacist handles deal with life and death, whereas commodities such as soap flakes and ores, although subject to analysis, have little or no direct bearing on the health of an individual. The scientists have given drugs to the people of the world; the pharmaceutical chemist guarantees the quality of these drugs now and in the future.

1.2 SELECTING THE SAMPLE

There are at least two good reasons for analyzing a drug or a drug in dosage form. First, it is one way (and often the only way) to judge quality. Second, any hazards associated with the drug can be ascertained and the necessary corrections made before the substance is marketed. These hazards should not be confused with the toxicity of the basic substance. The pharmaceutical analyst cannot control them, but he can assure the physician that the basic substance does not contain degradation products that might produce secondary toxicity when administered. For example, there is a basic risk involved in the administration of tetracyclines, and the physician must be aware of this when he decides to prescribe the drug. However, it is known that certain tetracyclines degrade to form epianhydrotetracycline and anhydrotetracycline, which have been shown to cause an acute syndrome associated with nausea, vomiting, proteinuria, glycosuria, acidosis, and aminoaciduria.[4,5] The pharmaceutical analyst must, therefore, guarantee that these substances are not present in the final dosage form. Regardless of the motives (and these, in certain instances, may be economic), the analyst must take a sample for analysis that will give him the most accurate picture of the composition of the whole batch. To go to the extreme, suppose a bottle of tetracycline has been exposed to heat and moisture for a brief period. The product is partially degraded, but mostly at the top of the bottle. If the analyst, by chance, removes a portion of the substance from the bottom of the bottle, the analytical results will show that the tetracycline is satisfactory, even though a portion is unfit for human consumption.

There has been much written about proper sampling procedures. The problem is essentially statistical in nature and, because of this, cannot be considered in detail in an elementary book on pharmaceutical analysis. Even if this were possible, many authorities claim that statistical treatments yield dubious results and place unwarranted work loads on the analyst. In its simplest form, sampling is an exercise in common sense, and, if only the generalities are considered, some statements may be made that will guide the analyst in choosing the proper number of units for analysis.

There are three main methods for obtaining samples. In *continuous sampling*, a certain portion of the material to be analyzed is continuously diverted to form the gross sample. For example, a water line may be tapped in such a way that small portions of water are continuously removed for analysis. In *intermittent sampling*, every *n*th unit is set aside to form the gross sample. At the production level, this may mean that every hundredth bottle is removed for examination. In most instances, however, the contents (either liquid or tablets) have already been analyzed, and this serves only as a final check on the product. *Grab sampling* is usually associated with homogeneous materials. A liquid, which has been thoroughly mixed, presents no particular problem, and this method can be used if the batch is not too large. In large-scale operations, it is preferable to sample the tank container at several depths, particularly if the product cannot be continuously stirred. Grab sampling may be used for heterogeneous materials, but a large number of samples should be taken from various parts of the batch to form the gross sample. This, then, is further subdivided to give the sample that is eventually examined in the laboratory.

Any or all of these methods may be used by the industry, depending on the product (or chemical). In the laboratory, however, the analyst is not acutely aware of sampling problems. He may be handed a bottle containing 100 acetylsalicylic acid tablets and asked to carry out a suitable analysis. The first step, as outlined in most pharmacopeias, is to count 20 tablets, weigh, and reduce to a fine powder. Twenty out of the 100 tablets is probably a representative sample, but that 100 tablets may have come from a batch of 10,000, 100,000, or perhaps even 1,000,000 tablets. Are the 20 tablets representative of batches of the sizes given above? Probably not, which is why total quality control is so important in the pharmaceutical industry.

The pharmacopeias do not spell out proper sampling procedures for all products. There is no doubt, however, that those who are responsible for the writing of these compendia are aware of these problems. The student, for example, should examine the sampling procedure given for vegetable drugs in the *U.S. Pharmacopeia*. The detail given there is indicative of the importance of taking a representative sample for analysis. One other aspect of sampling should be examined in detail. Many analytical procedures and

the weight variation test for uncoated tablets specify that 20 tablets be taken from the batch for examination in the laboratory. The USP weight variation test is given below.

> Weigh individually 20 whole tablets, and calculate the average weight; the weights of not more than two of the tablets differ from the average weight by more than the percentage listed in Table 1.2, and no tablet differs by more than double that percentage.

TABLE 1.2: Weight Variation Tolerances for Uncoated Tablets*

Average weight of tablet, mg	Percentage difference
13 or less	15
13 through 130	10
130 through 324	7.5
More than 324	5

* Reproduced, with permission, from the *U.S. Pharmacopeia.*

The purpose of this test is to control tablet weight and to ensure that no tablets deviate from a mean or average weight by more than is necessary. There is, however, some concern that the sample size is too small in relation to the number of tablets being manufactured (and this, in certain instances, may run into the hundreds of thousands). Many manufacturers realize this and run continuous weight checks on groups of tablets as compression is being carried out. The weight variation test is then used as a final check on the batch. Another alternative has been suggested. This is based on the observation that 20 tablets do not constitute an adequate sample and that it would be preferable to increase the sample size to prevent unsuitable batches from reaching the market. Although it is not too difficult to weigh 20 tablets individually, the handling of 50 or 100 tablets may present the analyst with an excessive workload. To decrease this workload, Dunnett and Crisafio[6] suggested a "two-sample" test which is based on 50 tablets, but the initial calculations depend on the individual weights of the first 20. If these 20 comply with their test criterion, the batch is accepted. If, however, more than one tablet deviates from the mean by more than 5 per cent, the remaining 30 tablets are weighed and the entire 50 are examined mathematically for uniformity. This type of test increases the probability of catching a bad batch of tablets and indicates the need for proper sampling procedures in quality-control operations.

Parallel to this is the actual analysis of these same tablets. It is too often assumed that tablets represent a "uniform" product. Each tablet in a batch varies slightly in both weight and potency. The label claim for the product

is an average value, and not all tablets contain the amount of drug stated on the label. To illustrate this point, assume that we have ten tablets; eight of these tablets contain 100 mg of active ingredient and the remaining two contain 50 and 150 mg, respectively. If these tablets are weighed and reduced to a fine powder and a sample is taken for analysis, the final result will indicate that each and every tablet contains 100 mg of drug. This is because we have destroyed the characteristics of the two defective tablets and averaged their deficiencies with the satisfactory tablets. Such a procedure tells us very little about the individual characteristics of the tablets and, according to some researchers, is an unsatisfactory approach to sampling and analysis.

We could conclude, on the basis of the analysis and weight variation test, that there is a direct relationship between tablet weight and tablet potency. Lighter tablets should contain less active ingredient; heavier tablets should contain more active ingredient. This, however, is not a valid conclusion, because it assumes uniform mixing of ingredients prior to compression. The work of Moskalyk et al.[7] emphasizes that the actual variability in drug dosage is, as a rule, greater than would be apparent from the variations in individual tablet weights. These researchers concentrated on tablets containing significant quantities of drug (0.5 g per tablet, on the average); hence the results are not too striking. However, they did quote from a paper that considered the same problem but dealt with tablets containing 5 mg ascorbic acid per tablet. The student will realize that it is much more difficult to mix a minute quantity of material uniformly into a large quantity of filler and disintegrant than it is to mix a powder containing a large percentage of active substance. In the example cited, the range of potencies was of the order of 15%. More thorough mixing decreased this value to 5.8%, but at no time was the potency directly related to the weight of the tablet.

The problems cited above are related to improper sampling. One suggested solution is to maintain a 20-tablet sample but to analyze these tablets individually. This involves an increase in the analytical workload and does not necessarily present a true picture of the total batch. Coupled to this is the basic analytical problem of determining drugs in individual dosage forms. Some drugs are so potent that they are administered in very small quantities, and the analyst cannot detect these quantities unless the sample size is increased. Tablet analysis is under review by several of the pharmacopeias, and it is probable that standards will be changed to include, where feasible, single-tablet analysis. This approach, together with a statistical treatment of the acceptance sampling of finished pharmaceutical products, is discussed in a paper by Breunig and King.[8]

The discussion in the preceding paragraphs has not given the student any specific solutions to the sampling of pharmaceuticals. It does, however, point out the hazards of assuming uniformity of a batch and basing analytical acceptance on a restricted group of tablets (or an isolated sample withdrawn from a large batch of powder, liquid, or suspension). Sampling complications

are endless and are related to statistical probability. The basis for proper sampling is, therefore, found in the mathematical courses dealing with error and probability.

Once the sample is selected, the analyst must process the product for analysis. These preliminary operations are usually simple, but the analyst must always keep in mind that the sample may react with something in the environment or decompose prior to separation and measurement operations. Samples should, therefore, be kept in tightly closed containers before and after preliminary treatment. Solid dosage forms must be reduced to a fine powder prior to further manipulation (usually done by using a mortar and pestle). The particle size of ground tablets should be of the order of 100 mesh, particularly if the product is coated. Large "chunks" of outer coating contain no active ingredient and can cause substantial analytical error. Various types of grinders and mixers are available to carry out these processes automatically, but few operating laboratories rely exclusively on such equipment. The proper sample, a good mortar and pestle, and a strong wrist are still the most satisfactory approaches to the analysis of the pharmaceutical dosage form.

1.3 SELECTING THE CHEMICALS

The pharmacist is familiar with a number of pharmacopeias and buys chemicals carrying a USP (*United States Pharmacopeia*), a BP (*British Pharmacopoeia*), or an NF (*National Formulary*) designation. There are, however, a number of other grades of chemicals that are superior or inferior in quality to those described in these compendia.

The specifications for USP chemicals are outlined in the current edition of that book. Tests are given for various impurities, and the maximum permissible amounts are stated. These specifications may be found in the main part of the text (that is, the chemical may have a monograph of its own) or in a separate section dealing exclusively with reagents, indicators, and solutions. Similar comments may be made for those chemicals carrying BP or NF designations.

A *CP-grade chemical* (that is, chemically pure) is purer than the commercial grades used in certain manufacturing operations, but its actual purity varies with the source and the type of substance.

Reagent-grade chemicals are used in many analytical operations and, in most instances, their specifications are those drawn up by the American Chemical Society. The letters "ACS" follow the name of the chemical, and the purity and use are often defined on the label of the marketed container.

Primary standard-grade chemicals are equal to and often superior to the ACS substances in quality. They approach 100% purity and are used as primary standards in volumetric analysis.

The pharmaceutical analyst normally uses USP or BP grade chemicals.

The reagent and primary standard grades tend to be more expensive and are reserved for special uses. It is important to remember that the compendia give minimum standards and that the actual purity approaches or is equal to that designated for the ACS grade. In addition to this, many analyses specify that a blank determination be performed on the reagents; this, in effect, corrects for the presence of impurities. When in doubt, use the best grade of chemical available.

Chemicals in containers should be handled in such a way as to prevent

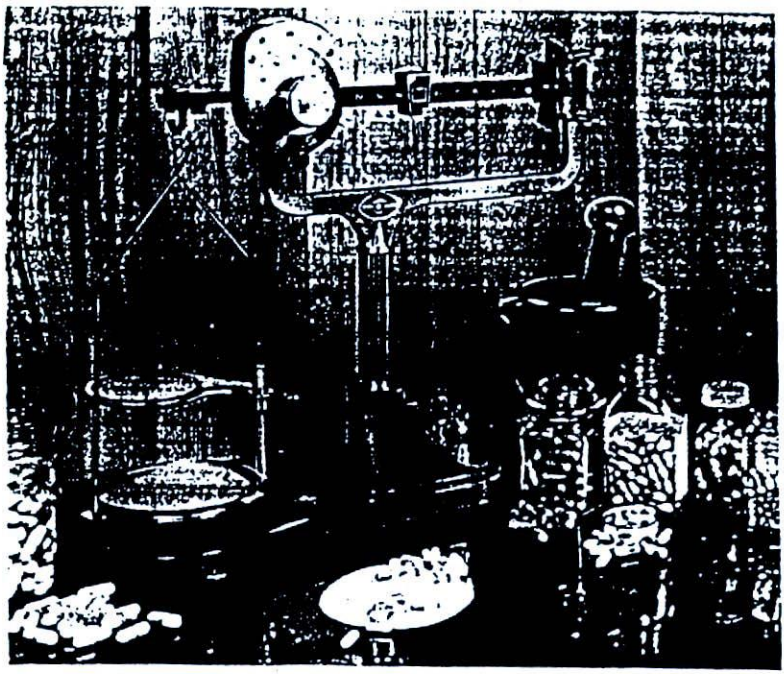

FIGURE 1.1: The chemicals used in the preparation of solutions may be weighed with sufficient accuracy on the Dial-O-Gram 310 balance, or its equivalent. (Courtesy Ohaus Scale Corporation, Union, N.J.)

contamination. If the container is large, a portion of the chemical should be transferred to a smaller bottle to facilitate handling in the laboratory. Caps or glass stoppers should never be placed in contact with contaminated surfaces. A glass stopper is best removed with the first and second fingers, with the palm of the hand facing upwards, and the same hand used to pour the contents of the container into another vessel. A cap may be removed with the small finger of the hand by holding it between that finger and the palm of the hand. This gives the analyst maximum flexibility in handling the

container, the cap, and a spatula, all at the same time. (It is obvious that this technique cannot be used if the container cap is too large to handle in the manner indicated above.)

In most analytical operations, the dry chemical is weighed and dissolved in a suitable solvent. In preparing most test solutions, it is not necessary to weigh the chemical on an analytical balance. A balance, such as that shown in Fig. 1.1, is sufficiently accurate for most purposes. Even though a precise weighing (that is, to four decimal places) is not required, the weighing should be carried out carefully and the chemical transferred quantitatively to the appropriate vessel. The solvent is then added to dissolve the chemical, and the solution is stored in tight containers made of resistant glass or other suitable material. Certain solutions should be stored in light-resistant containers, and any pharmacopeial directions in this regard should be carefully followed. If water is the solvent, it should be free of impurities and if USP procedures are being followed, *purified water* must be used in all tests and assays. This water is obtained by distillation or deionization, and the specifications are outlined in the monograph in this compendium.

1.4 MEASUREMENT BY MASS

The postwar years have been called, among other things, the Atomic Age, the Age of the Computer, the Age of the Tranquillizer, and, most recently, the Space Age. These "Ages" have one thing in common—instruments by the thousands. The student will approach one of these newer analytical instruments with awe, handle it with the utmost of care, and extol its virtues, particularly if its cost has catastrophically affected the institutional budget for several years. The analytical balance, on the other hand, receives no special praise, is handled with reasonable care (but mostly at the insistence of the instructor), and rarely costs more than a few hundred dollars. It is unfair to compare instruments, because each has its own unique place in the analysis of the commodities desired by society. However, the analytical balance is in a category by itself. It is *the* basic tool of pharmaceutical analysis and provides a precision that is not attained by most other measuring instruments. For example, what instrument is capable of readily differentiating between two samples weighing 100.0000 and 100.0001 g? Most scientists would be hard pressed to give any answer other than "the analytical balance." It, therefore, deserves the respect it so rarely gets, and it must be used properly, regardless of of the nature of the weighing being undertaken by the student.

A. MASS AND WEIGHT

Mass is a fundamental property of matter. It is independent of acceleration or velocity (except that an increase in mass is observed near the velocity of

light) and it is unaffected by its position with relation to the surface of the earth. *Weight*, on the other hand, is a measure of the gravitational effects on a given object.

The force on an object is equal to the product of the mass and the acceleration (Newton's second law of motion). Weight (w) is equal to the product of the mass (m) and the acceleration of gravity (g). The last quantity varies at different locations on the earth's surface, and therefore, the weight of the object will also vary. The mass, however, does not change.

It is customary to speak of the "weight" of an object, and this is acceptable because the process of weighing involves a comparison of a standard with the object. The weight of the standard is given by Eq. (1.1). The weight of an

$$w_1 = m_1 g_1 \tag{1.1}$$

object is as in Eq. (1.2). At equilibrium, on an analytical balance, $w_1 = w_2$,

$$w_2 = m_2 g_2 \tag{1.2}$$

and $m_1 = m_2$, since the acceleration of gravity affects both pans of an equal-arm balance in the same way. The ratio of the weights is the same as the ratio of the masses, and the words "weight" and "weighing" may be used in our discussions of the measurement of the mass.

B. THE BALANCE

A modern analytical balance is shown in Fig. 1.2. A central column is fastened to the base of the instrument. A plane agate (or sapphire) bearing is built into the top of this column, and the knife-edge agate bearing of the beam is placed in contact with it. The beam itself is made of some suitable alloy and is usually graduated in milligrams or grams. Knife-edge bearings are located at the ends of the beam, and pans are suspended downward via a stirrup hook. A pointer extends from the center of the beam to a graduated scale attached to the base. This scale is movable over a short distance and is graduated, beginning with zero in the center, into 10 or 12 units to the left and to the right. Pan and beam-arrest knobs are located in the base of the instrument. Auxiliary parts, such as the rider knob, are indicated on the illustration.

Balance design involves a compromise between the factors that affect the sensitivity of the instrument. *Sensitivity* is usually defined as the number of scale divisions the rest point is displaced by a load of 1 mg. It has also been defined as the fraction of a milligram required to produce a displacement of one scale division. The one value is the reciprocal of the other. The factors affecting the sensitivity of the analytical balance are inherent in Eq. (1.3). The

$$\tan \alpha = \frac{lk}{dm} \tag{1.3}$$

tangent of the angle through which the pointer moves (tan α) is equal to the product of the length of the beam (l) and a constant for the coefficient of friction for the system (k) divided by the product of the mass of the beam (m) and the distance between the center of gravity and the knife-edge at the top

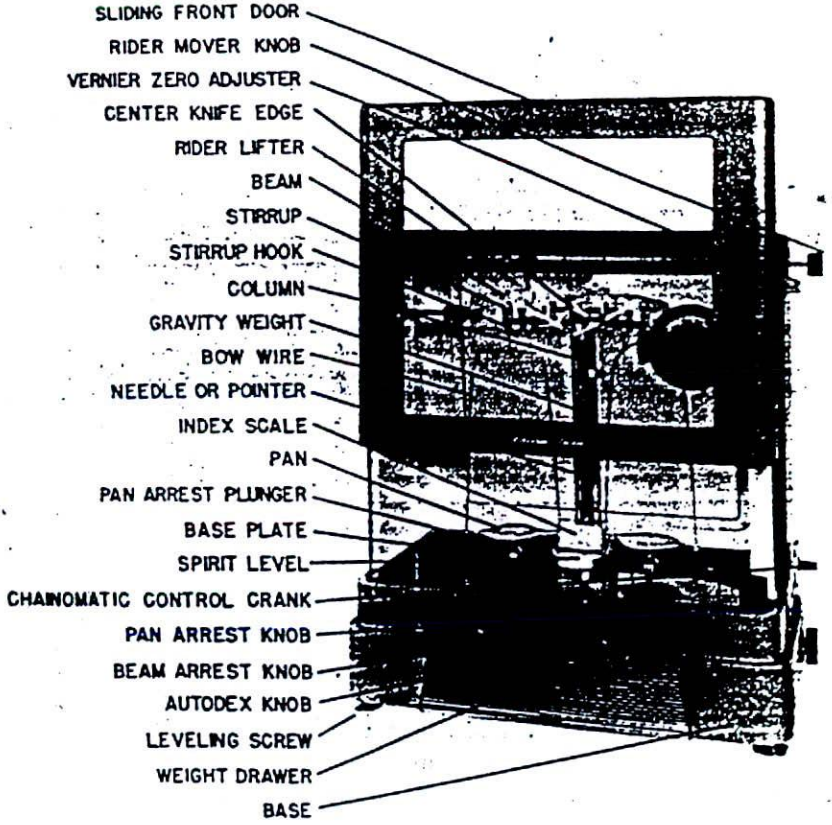

FIGURE 1.2: The Analytical Balance and Its Principal Parts. (Courtesy of the Torsion Balance Company, Clifton, N.J.)

of the central column (d). A longer beam, a lighter beam, and a decrease in the distance between the knife-edge (the fulcrum of rotation) and the center of gravity will increase the sensitivity of the balance.

It is impossible to lengthen and lighten a beam at the same time. A long thin beam could not be incorporated into a reasonably sized cabinet and would not be able to support the loads normally encountered in the weighing operation. Even under normal conditions, the beam will give slightly as the load on the pans increases. This implies that the sensitivity of the analytical

balance decreases as the weight on the pans increases. Therefore, the balance will have a load limit, which is usually considered that amount of weight which reduces the balance sensitivity to approximately one-half that observed when no weights are in position on the pans. It is usually customary to determine the sensitivity at various loads and then to construct a sensitivity curve (divisions per milligram versus load in grams).

The center of gravity may be controlled, in part, by adjusting a weight located on the pointer of most analytical balances. If the center of gravity is higher than the fulcrum, the beam will not stay in position. If the center of gravity coincides with the rotation point, the beam will remain in one position. As the distance between the fulcrum and the center of gravity increases, and this implies that the center of gravity is below the fulcrum, the time of swing of the pointer becomes more rapid and the balance sensitivity decreases. Most analytical balances are so adjusted that the center of gravity is approximately 0.2 mm below the fulcrum.

Under normal operating conditions, the modern analytical balance has a sensitivity of approximately three divisions per milligram. If the value is less than this, the balance should be checked and adjusted by a knowledgeable person.

C. TYPES OF BALANCES

Equipment catalogs illustrate hundreds of different types of analytical balances. Many of these are sold to laboratories involved in specialized measurements (for example, remote-control balances are used in laboratories handling radioactive isotopes) and are not normally used by the pharmaceutical analyst. However, all weighings consist of comparing one value with another known value, and this can be done by utilizing a calibrated force, usually a set of calibrated weights but, in certain types of balances, a force based on elastic or electrical properties.

A torque balance is illustrated in Fig. 1.3. The principle of operation is outlined in Fig. 1.4. The manufacturers of such balances usually produce 10 or 12 types covering the various weight ranges desired by the analyst. For the balance illustrated, it is possible to obtain types that will weigh in the 0 to 1-mg range, the 0- to 10-g range, and in various ranges between these values.

An *electrobalance* (for example, the Cahn Electrobalance) is also a microbalance in that it is used to weigh microquantities of material. In principle, it is similar to the balance described above except that the balance beam is lined up by controlling current through a coil with a potentiometer. Since the beam is always returned to the same position, torque is proportional to current. The instrument is battery-operated and is, therefore, completely portable.

By far the most common type of balance is that described in Section 1.4B. The various subtypes in this group differ only in external construction and in the method by which the calibrated weights are handled.

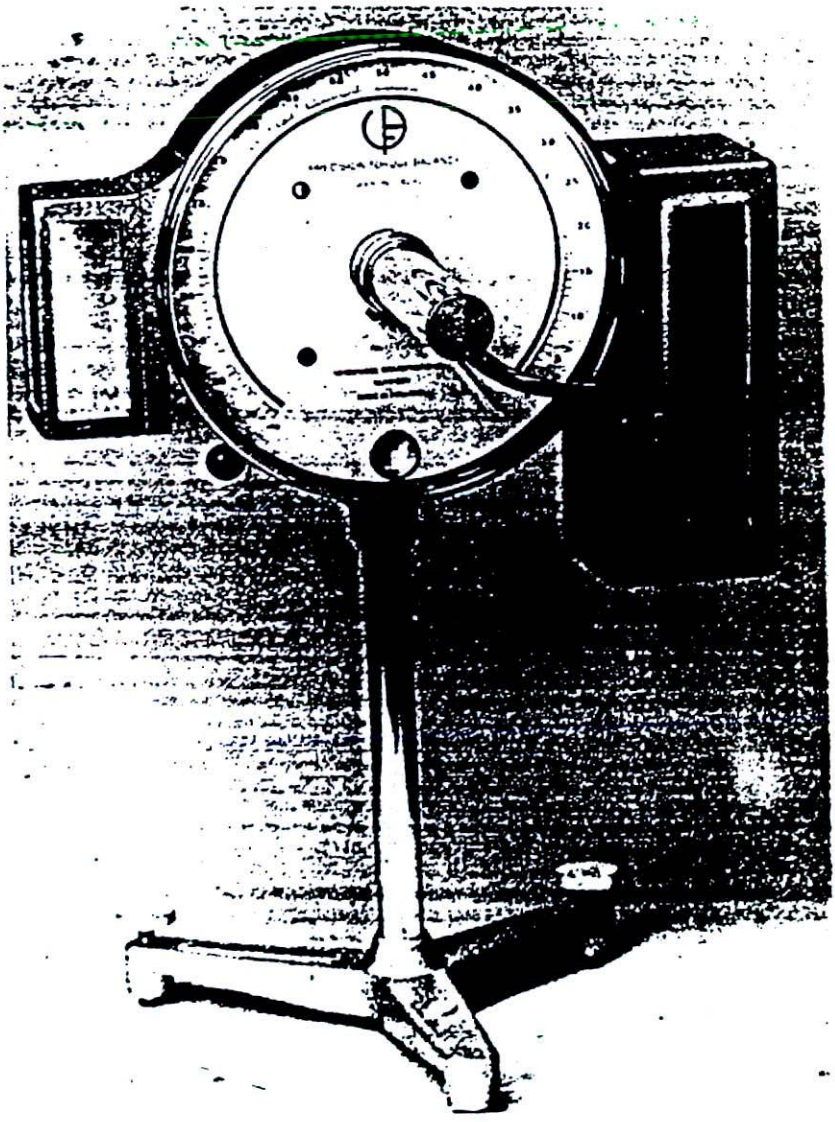

FIGURE 1.3: The VDF Micro-Torque Balance. (Courtesy of Canadian Laboratory Supplies Ltd., Montreal, P.Q., and the manufacturer.)

It is relatively easy to handle weights in excess of 100 mg. Small weights present more of a problem, and methods have been devised to apply these to the pan or the beam or to allow for the use of some secondary system that can be calibrated in terms of weight. The simplest approach is the use of a rider. This may be a 10-mg rider or a 1-g rider such as that shown in Fig. 1.2. The

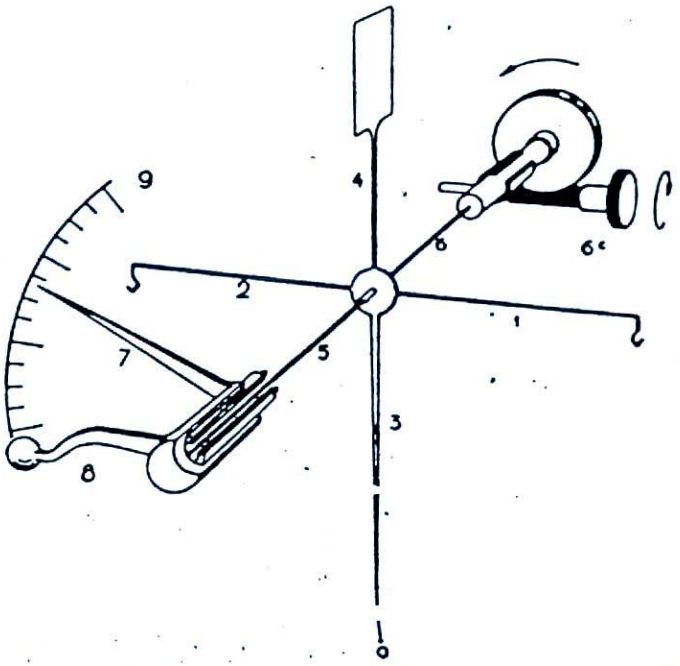

FIGURE 1.4: Operating Characteristics of the VDF Micro-Torque balance. The beam, with its branches 1, 2, 3, and 4, is fixed to the torsion wire (5, 6), which also serves as a suspension wire. If a load is hung on branch 1, branch 3 moves away from zero. Equilibrium is restored by moving lever 8 until branch 3 is again at zero. The weight pointer (7) shows how much the wire has been twisted. Since this is a measure of the suspended load, the scale (9) is subdivided into units of weight. The weight read on the scale is added to that of the load on branch 2 to determine the weight on branch 1. Branch 4 represents an air-damping system, and this prevents excessive oscillation of branch 3 around the zero point. (Courtesy of Canadian Laboratory Supplies Ltd., Montreal, P.Q., and the manufacturer.)

rider sits on top of the beam and, when moved from left to right, the weight on the pan is altered. Another method is to use a chain hung from the beam to an adjustable point on a drum or a post. By this method, the effective length of the chain can be varied, and this can be calibrated into the appropriate number of units. Figure 1.2 is an example of a *chainomatic balance*.

A third approach is based on beam deflection. Within limits, beam rotation is proportional to weight, and this principle is used in the *projection balance*. Such a balance is illustrated in Fig. 1.5. As the beam rotates, the pointer

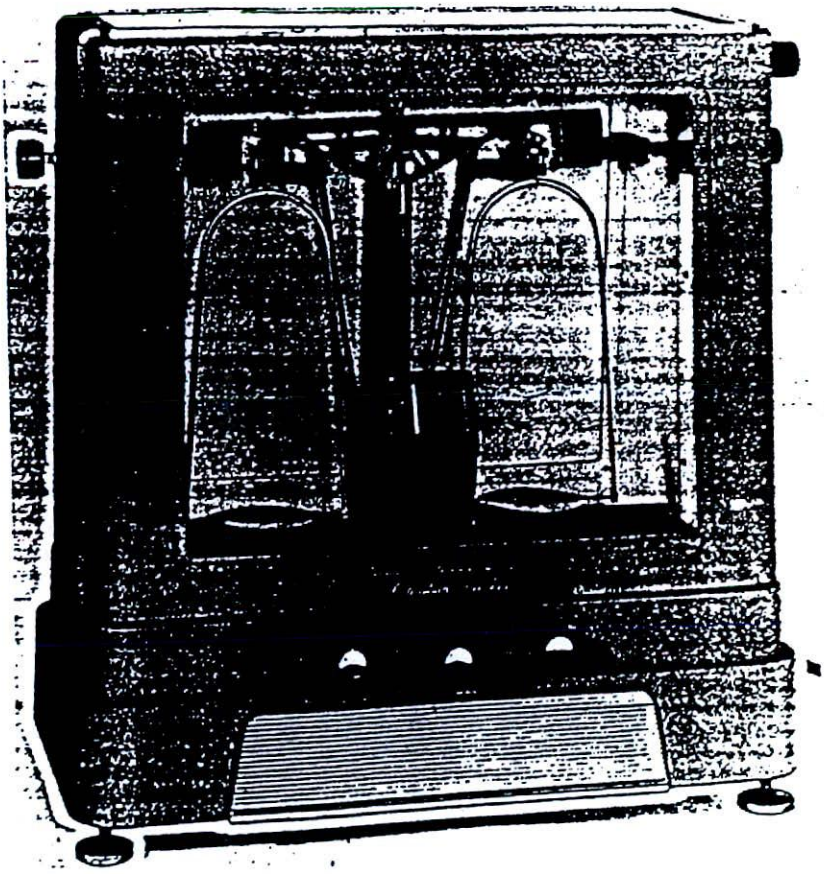

FIGURE 1.5: The Christian Becker Projectomatic Balance, Model AB-1. (Courtesy of the Torsion Balance Company, Clifton, N.J.)

rotates, and the weight can be read off the dial located in front of the central support column.

No one method can be used exclusively if the analytical balance is to attain a maximum of accuracy. Therefore, all balances use mixed systems, that is, weights and rider; weights, rider, and chain; or weights and deflection.

One of the more popular types of balance is that illustrated in Fig. 1.6. Several manufacturers now produce these single-pan balances. The weights

may be added to or removed from the beam, depending on the manufacturer. The latter principle is used in the true substitution type of balance. The balance operates at full load at all times. The calibrated weights are counterbalanced by an equal weight on the other end of the beam. An object on the

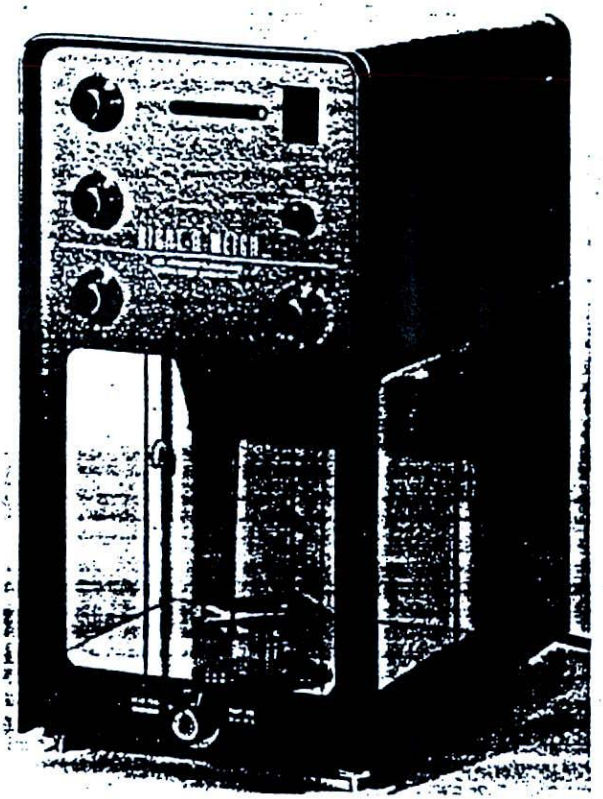

FIGURE 1.6: The Ainsworth Right-A-Weigh Balance. (Courtesy of W. Ainsworth and Sons, Inc., Denver, Col.)

pan will disturb equilibrium, and weights must be removed, by turning knobs, to bring the instrument into balance. Second, third, and fourth decimal places are determined via the projection principle. The analyst reads directly from a scale calibrated in units of 1 mg. For the balance illustrated, this scale is located in the upper right corner of the front panel. Because these balances operate at constant load, they have a constant sensitivity. They are also well-adapted to the beam-deflection principle. In addition to this, the single-pan balance has no arm-length error. A standard analytical

balance must be constructed so that both arms of the beam are of equal length. If the distance between the fulcrum and the pan on one side is not the same as that on the other side, an error in weighing will result. In a single-pan balance, the weighing is performed on the same side that carries the calibrated weights. This, in effect, eliminates this error, because both the weights and object act on the same arm of the beam.

D. WEIGHING

When the beam and pans of an analytical balance are released, the pointer will begin to move across the face of the scale at the bottom of the support column. If the balance is not disturbed, the pointer will eventually stop swinging, and the rest position on the scale can be read. A maximum of patience is the basic requirement for determining the zero point of the balance in this manner, and the method is not highly recommended.

Most instrument manufacturers are prepared to aid the harassed analyst and have, therefore, built damping devices into certain types of analytical balances. The purpose of these devices is to stop the swing of the pointer and, in this way, to speed up the weighing process. Air chambers are used as damping devices in certain types of single-pan balances (for example, the Ainsworth Right-A-Weigh), but the magnetic device is still the simplest and cheapest way to stop pointer movement. A magnet is usually built into the balance at the same level as the beam. One end of the beam, for example, the left end, is made of a wide plate of aluminum, and it is this that moves between the poles of the magnet. The device is further refined by attaching the magnet to a rod. When the rod is moved outward, the poles of the magnet no longer affect the aluminum plate and the pointer of the balance is again free-swinging.

To weigh an object on a single-pan balance, the student must be able to rotate knobs and read numbers accurately. Both operations have been known to confuse some students. To weigh an object on a balance such as that shown in Fig. 1.5, the student is faced with certain additional hazards. First, weights must be removed from a weight box and transferred to the right pan. Second, the student must be able to add, since the projection principle is unable to cover the entire weight range. To weigh an object on an analytical balance that is neither damped nor calibrated on the index scale, the student must possess the skills of a jeweler (for example, small weights must be handled and riders shifted carefully along the beam) and a mathematical ability involving the processes of counting, addition, and division.

It is at once simple and tedious to weigh an object on a standard analytical balance. To carry out such a weighing, we must consider the weights placed on the proper pan, the position of the rider on the beam, and the information obtained from the index scale of the analytical balance. Let us assume that the balance at our disposal is of the rider type and that the beam is calibrated,

from the center outward, to a maximum of 10 mg. By moving the rider along the beam, we can add anywhere from 0 to 10 mg to the effective weight on the right-hand pan. The index scale is divided into 20 equal units and is marked with a zero in the center. There are, therefore, 10 equal units to the left and 10 equal units to the right of zero. The scale can be considered plus (to the right) and minus (to the left).

With the pans empty, the equilibrium position of the pointer is called the *zero point*. To determine this point, the pointer is set in motion and a series of readings are taken on the index scale. It is essential that an odd number of readings be taken on one side of the zero and an even number on the other side, because the amplitude of the pointer swing decreases with each sweep across the index scale. Usually, three readings are taken to the left and two to the right of zero. An example is given below.

	Left	Right
	−6.4	
	−6.0	+5.6
	−5.6	+5.2
average	−6.0	+5.4

The zero point is

$$\frac{-6.0 + 5.4}{2} = -0.3$$

This means that the zero point is −0.3 units to the left of zero on the index scale. In recording the readings, the decimal place must be estimated, and it is customary to round off all values to one decimal. It is possible to reproduce the value obtained for the zero point to within 0.2 to 0.3 units.

The rider is now moved to the 1-mg mark on the beam, and the above process is repeated. The point located by this process is called the *rest point*. Let us assume for our purposes that this point is −4.3. This means that a 1-mg weight has moved the pointer four units. In other words, each unit is equal to 0.25 mg. We have determined the sensitivity of the balance.

Sensitivity, however, depends on the amount of weight on the pans. The value determined above is valid for an empty balance and cannot be used if maximum weighing accuracy is desired. Sensitivity must, therefore, be redetermined with weights on the pans of the balance. The procedure is identical to that described above, except that the zero point and the rest point are determined with equal weights on both pans. A 5-g weight is placed on the left pan, and a 5-g weight is placed on the right pan. After the sensitivity at this level has been determined, the 5-g weights are replaced by 10-g weights, and so forth, until the maximum capacity of the balance is reached. A curve of divisions per milligram versus load in grams is now plotted and permanently retained by the analyst.

It is now possible to weigh an object accurately. The object is carefully positioned on the left pan of the balance. Weights are carefully removed with forceps from the weight box and transferred to the right pan. If the weight of the object is a complete unknown, it can be approximately weighed by using a balance such as that shown in Fig. 1.1. This serves as a guide to the transfer of the proper number of weights to the right pan. Let us assume that the weights on this pan are 5, 2, and 2 g. We have also added a 500- and a 50-mg weight. The balance is still not in equilibrium, and we must add 2 mg more by using the rider on the beam. At this point, the balance is more or less in equilibrium. The rest point is now determined and found to be −1.8. The total weight on the pan is 9.552 g and, on the basis of the rest point, this is slightly more than the weight of the object. The original zero point for the balance was −0.3, and this means that we are still 1.5 units away from this point. Weight must be removed from the right pan if we are to obtain the true weight of the object. This, of course, is too tedious, and the amount of weight that must be removed can be readily calculated. Let us assume that our sensitivity curve indicates a value of 0.2 mg per unit at this load level. It is necessary, therefore, to remove 0.2 × 1.5 or 0.3 mg. The weight of the object is thus 9.5517 g.

This procedure for the determination of zero and rest points is called the *method of swings*. Even though other methods are described in the scientific literature, it is the method of choice. The *method of short swings* is much the same as that described, except that the pointer is not allowed to swing more than 2 or 3 units in either direction and only two readings are taken on either side of zero. This is permissible, since the decrease in amplitude of pointer swing under these circumstances is small and the average of the two readings is very close to the true zero point.

E. METHODS OF WEIGHING

An object can be weighed on an equal-arm analytical balance in a variety of ways.

1. Direct Weighing

The object is placed on the left pan, and the weights are placed on the right pan of the analytical balance. The analyst will use this method for weighing most of the materials used in the laboratory.

Many of the liquids and powders used by the pharmaceutical analyst are stored in weighing bottles. A bottle and its contents are weighed, a portion of the contents is removed, and the bottle and its contents are reweighed. The difference in weights is equal to the weight of the contents removed from the bottle. This is known as *weighing by difference*, and is a standard procedure in many laboratories. If this method is used, it is not necessary to know the

FIGURE 1.7: Handling weighing bottles with crucible tongs (left) or strips of paper (right).

zero point of the balance. It is sufficient to consider the rest point obtained during the first weighing the zero point. Each individual reading is in error, but the error is the same in both instances and cancels out when the subtraction is made. This assumption is valid only if the two weighings are carried out within minutes of each other.

Two methods of handling weighing bottles are shown in Fig. 1.7.

2. Transposition Weighing

The method of transposition weighing involves two direct weighings. The object is weighed in the manner indicated above; then the object and weights are interchanged, that is, the object is placed on the right pan and the weights on the left pan.

On the basis of the principle of moments. Eqs. (1.4) and (1.5) are valid:

$$ML_1 = W_1L_2 \tag{1.4}$$

and

$$ML_2 = W_2L_1 \tag{1.5}$$

Multiply Eq. (1.4) by Eq. (1.5) and solve for M.

$$M = \sqrt{W_1W_2} \tag{1.6}$$

The terms in Eqs. (1.4)–(1.6) are mass (M), length of the left arm of the balance (L_1), length of the right arm of the balance (L_2), weight when the object is on the left pan (W_1), and weight when the object is on the right pan (W_2).

This method of weighing compensates for any inequalities in the lengths of the arms of the beam.

3. Differential Weighing

Differential weighing is used when a small change in a large quantity must be determined. For example, the analyst may be determining the amount of carbon dioxide taken up in one of the absorption tubes of a CO_2 train. The weight increase in such a tube may be relatively small. The usual procedure is to place a similar tube on the right pan instead of weights. A "true tare" is obtained and any errors due to the tube will cancel out. The small weight increase can now be determined with a much greater accuracy.

4. Substitution Weighing

In substitution weighing, the object is first counterbalanced with spare weights or lead shot. The object is removed, and weights are added to the left pan until the same rest point is obtained. The zero point of the balance need not be known. As in transposition weighing, Eqs. (1.7) and (1.8) are

valid. T is the weight of the tare substance. If Eqs. (1.7) and (1.8) are divided through by L_1, it becomes obvious that W_1 is equal to W_2.

$$TL_2 = W_1L_1 \tag{1.7}$$

$$TL_2 = W_2L_1 \tag{1.8}$$

A weighing can also be carried out by using the principle inherent in the single-pan balance. First, equal weights are placed on both pans. The object is placed on one pan, and weights are removed from that pan until the original rest point is obtained. The weight of the object is thus equal to the weights removed from the pan.

This method of weighing eliminates arm-length errors in exactly the same way as does the single-pan balance. The object and the significant weights act on the same side of the balance and hence on the same arm of the beam.

F. WEIGHING ERRORS

The balance will provide the analyst with the most accurate results. However, errors do occur, and the analyst must keep these in mind when weighing an object on an analytical balance.

1. Errors from Carelessness

It is relatively easy to handle weights and objects with the finger tips but somewhat more difficult to accomplish the same manipulations with forceps and crucible tongs. The latter technique is recommended.

2. Instrumental Errors

The balance should be checked from time to time to ensure that it is operating at maximum efficiency. Unequal balance arms contribute to weighing errors, but the analyst cannot control balance construction. He can, however, use the methods previously described to correct for these arm inequalities.

3. Weight Errors

Analytical weights may become damaged after years of use and can significantly affect the results.

4. Buoyancy Errors

Archimedes' principle states that an object is buoyed up by the weight of air it displaces. In the weighing process, the object and the weights do not displace the same amount of air and, if a maximum of accuracy is desired, corrections must be made for the differences in density of the object and the

analytical weights. In most analyses, the density of the weights is greater, and the object displaces a greater volume of air than do the weights. The true weight of an object is, therefore, more than that observed under normal conditions. Equation (1.9) relates the various factors involved to the true weight of the object in vacuum. W_t is the true weight, W_a is the apparent

$$W_t = W_a + W_a\left(\frac{0.0012}{D_0} - \frac{0.0012}{D_w}\right) \tag{1.9}$$

weight, D_0 is the density of the object, D_w is the density of the weights, and 0.0012 is the density of air under normal conditions.

Buoyancy errors are small and are not considered in most analyses. Certain techniques, such as the calibration of volumetric glassware, do require the application of Eq. (1.9).

5. Errors from Atmospheric Moisture or Carbon Dioxide

Certain substances will readily absorb moisture or carbon dioxide. These materials will increase in weight during the weighing process and must be protected by using closed containers or by keeping the balance case as dry as possible with substances such as silica gel.

High humidity can significantly change the weight of large objects (for example, volumetric flasks). Water may be absorbed directly onto the glass surface and thus increase the weight of the object. Under such circumstances, the object should be weighed by the *differential* method.

6. Errors from Temperature Changes

The object and the balance should be at the same temperature when the weighing is carried out. If the temperature of the object is greater than that of the balance, an upward current of air will result and cause a negative weighing error. If the object is colder than the surrounding balance parts, a positive error will occur. It has been reported that a 5° difference between crucible and balance pan will cause a 1-mg error in weight. It is essential, therefore, that all heated (or cooled) objects should be returned to room temperature in a desiccator before a weighing is attempted.

7. Static Electricity Errors

Static electricity errors occur in very dry climates or in balance rooms that have dry, artificially controlled atmospheres. The static charges on glass containers and fine powders can cause erratic beam movement. The charge on glassware can be dissipated by wiping the surface with a damp cloth or by placing a weak alpha emitter in the balance case. The latter radioactive sources are available from a number of laboratory supply houses.

G. ANALYTICAL WEIGHTS

The National Bureau of Standards in Washington, D.C., divides analytical weights into two categories.

1. Precision Laboratory Standards.

This division includes Class M, Class S, and Class S-1 weights. Class M weights are the best available and are of one-piece construction. The other class weights may consist of a base into which a handle is screwed.

Good analytical weights are made from a stainless alloy with a high nickel-chrome content. Many whole-gram weights are made of bronze and coated with rhodium, gold, platinum, or a thin layer of lacquer. Milligram to gram weights are usually made from aluminum or tantalum.

2. Laboratory Weights

Class P and Q weights are considered laboratory weights. These weights are not as accurately made as those described above, but they can be used in routine analytical work.

Even the best weights have certain permissible tolerances. The National Bureau of Standards describes these in detail in Ref. 9, but, as an example, let us consider a 1-g weight. The allowable tolerance for the Class M weight is 0.034 mg; for the Class S weight it is 0.054 mg; for the Class S-1 weight, 0.10 mg; and for the Class P weight it is 0.2 mg. In exactly the same order, the tolerances for a 10-mg weight are 0.0054, 0.014, 0.030, and 0.060 mg. From these few values, it is evident that the Class M weights have the tightest tolerances and are, therefore, reserved for the calibration of other weights or for precise analytical work.

Few students and fewer analysts ever calibrate analytical weights—their accuracy is seldom questioned. The main reason for this is that reasonably accurate weights are available from reputable suppliers at moderate prices. Old weights can be discarded and replaced with new sets without spending much money. It should be noted, however, that even a reasonably good set of weights may cost $50, and the best Class M weights are priced in the $400 to $500 range.

The National Bureau of Standards will calibrate a set of weights for a fee, and this set can then be used to calibrate other sets of weights in the laboratory. Each piece in the set is checked against the corresponding piece in the calibrated set, and the deviation from the true value is noted. The best balance and the substitution method of weighing should be used when weights are calibrated.

Some laboratories do not have access to a good set of calibrated weights and will, therefore, rely on the relative method of calibration. In this method, one weight, for example, the 1 g weight, is assumed to have a certain precise value, in this instance, 1.0000 g. Each of the other pieces in the set is then

calibrated with respect to this standard weight. The weights so calibrated will not yield the absolute weight of the object. In most analytical operations, this is not too critical, because the final results are based on a ratio of weights. Moreover, minor errors within the individual weights will not critically affect the final answer.

The relative calibration method is too time-consuming to be used routinely. The absolute method is preferable, and most laboratories can obtain, if only for a few hours, a good set of analytical weights and use these as primary standards in the calibration process.

1.5 MEASUREMENT BY VOLUME

The volumetric flasks, pipettes, and burettes used in the laboratory are made from either borosilicate or soda-lime glass. The former glass has a low coefficient of expansion, and this lends itself to the construction of flasks with heavy walls and, therefore, with a high resistance to thermal shock. Few chemicals attack this glass and, since it contains a restricted number of elements, it will not contaminate the liquids in contact with it. Vessels to be used at or near room temperature are made from soda-lime glass. Most common reagents and titrants used in the laboratory will not appreciably attack this type of glass.

All volumetric containers must be appropriately marked to indicate total or partial capacities. Capacity lines are produced by etching with hydrofluoric acid, by engraving with an abrasive blast, or by applying a staining material, heating, and, in this way, imparting a color to the glass. Etched or engraved lines may be colored by staining or by using a special glass enamel.

Most volumetric glassware is also marked to indicate method of use, that is, TC (to contain) or TD (to deliver). Certain pipettes are calibrated to deliver a specified volume when the liquid remaining in the tip is blown out and added to that previously removed. These "blowout" pipettes are marked with an opaque band about $\frac{1}{8}$-in. wide well above the zero-milliliter capacity line.

A. CALIBRATION OF VOLUMETRIC APPARATUS

The accuracy of the volumetric apparatus used in the laboratory is rarely questioned by the pharmaceutical analyst. He has faith in this apparatus because most glassware is supplied by a few major manufacturers and meets tolerances similar to those established by the National Bureau of Standards. These, or similar tolerances, are accepted by the pharmacopeias and published in the appropriate appendices. Examples of such capacity tolerances are given in Table 1.3.

Volumetric flasks, pipettes, and burettes are calibrated at 20°C, and, although they are used at temperatures a few degrees higher than that specified,

the actual error introduced into the analysis as a result of volume change is inconsequential. However, under certain circumstances, it is important to know the exact capacity of the volumetric flask, pipette, burette, or newly constructed piece of apparatus. The analyst must then resort to one of several calibration procedures described in the scientific literature.

Calibration is usually carried out by weighing the quantity of water or mercury in, or delivered from, the particular piece of volumetric glassware. Distilled water is used in most testing methods, and the techniques described herein are based on this solvent.

TABLE 1.3: Capacity Tolerances for Volumetric Apparatus*

Volumetric flasks			Transfer pipettes			Burettes		
Designated volume, ml.	Limit of error, ml.	Limit of error, %	Designated volume, ml.	Limit of error, ml.	Limit of error, %	Designated volume, ml.	Subdivisions, ml.	Limit of error, ml.
10	0.02	0.20	1	0.006	0.60	10 ("micro" type)	0.02	0.02
25	0.03	0.12	2	0.006	0.30	25	0.10	0.03
50	0.05	0.10	5	0.01	0.20	50	0.10	0.05
100	0.08	0.08	10	0.02	0.20			
250	0.12	0.05	25	0.03	0.12			
500	0.15	0.03	50	0.05	0.10			
1000	0.30	0.03	100	0.08	0.08			

* Reprinted, with permission, from the *U.S. Pharmacopeia*. See also, *Natl. Bur. Std. (U.S.), Circ.* 602, April 1, 1959.

The true capacity of a piece of volumetric glassware at 20°C can be obtained from the apparent weight of the distilled water contained or delivered at any other temperature. It is necessary, however, to take into account the buoyancy effect of the air on the water and the weights, the change of density of the water, and the change of volume of the glass with temperature. Equation (1.8) is the basic equation that corrects for the buoyancy effect and the density of water. This equation provides the analyst with the weight of water in vacuum but does not take into consideration the change in glass volume with temperature. Equation (1.10) relates the various quantities to

$$V_0 = V_t + V_t\alpha(T_0 - T_t) \qquad (1.10)$$

the capacity at 20°C. V_0 is the capacity at the standard temperature T_0; V_t is the capacity at the weighing temperature T_t; and α is the cubic coefficient of expansion of glass. The last value varies with the type of glass used in the construction of the volumetric glassware.

Although the capacity may be calculated by using the equations cited, it is more common to use calculated data such as that shown in Tables 1.4 and 1.5.

TABLE 1.4: Corrections to be Added to the Apparent Weight of Water in Grams to Obtain Volume at 20°C of Glass Vessels Made of Borosilicate Glass*

Temperature, °C	Tenths of degrees									
	0	1	2	3	4	5	6	7	8	9
15	0.200	0.201	0.202	0.204	0.205	0.207	0.208	0.210	0.211	0.212
16	.214	.215	.217	.218	.220	.222	.223	.225	.226	.228
17	.229	.231	.232	.234	.236	.237	.239	.241	.242	.244
18	.246	.247	.249	.251	.253	.254	.256	.258	.260	.261
19	.263	.265	.267	.269	.271	.272	.274	.276	.278	.280
20	.282	.284	.286	.288	.290	.292	.294	.296	.298	.300
21	.302	.304	.306	.308	.310	.312	.314	.316	.318	.320
22	.322	.324	.327	.329	.331	.333	.335	.338	.340	.342
23	.344	.346	.349	.351	.353	.355	.358	.360	.362	.365
24	.367	.369	.372	.374	.376	.379	.381	.383	.386	.388
25	.391	.393	.396	.398	.400	.403	.405	.408	.410	.413
26	.415	.418	.420	.423	.426	.428	.431	.433	.436	.438
27	.441	.444	.446	.449	.452	.454	.457	.460	.462	.465
28	.468	.470	.473	.476	.479	.481	.484	.487	.490	.492
29	.495	.498	.501	.504	.506	.509	.512	.515	.518	.521
30	.524	.526	.529	.532	.535	.538	.541	.544	.547	.550
31	.553	.556	.559	.562	.565	.568	.571	.574	.577	.580
32	.583									

* (Nominal capacity, 100 ml) All values based on cubical coefficient of expansion of 0.000010 ml/ml/°C. The values in this table are based on Kimble KG-33 glass (Kimax) and are reprinted through the courtesy of the Owens-Illinois Glass Co., Toledo, Ohio, from the booklet *The Care and Handling of Glass Volumetric Apparatus.*

The values therein correct for the buoyancy effect, for water density, and for glass expansion. The following example illustrates the use of Table 1.4, which refers only to glassware constructed from borosilicate glass.

nominal capacity of vessel	25 ml
weighing temperature	22.5°C
weight before filling receiver*	24.964 g
weight after filling receiver*	0.044 g
apparent weight of water at 22.5°C	24.920 g
correction for 25 ml at 22.5°C (0.25 times value in Table 1.4)	0.083
volume of vessel at 20°C	25.003 ml

* Vessel weighed by method of substitution.

Certain factors must be considered during the actual calibration of the volumetric glassware. The weights should be calibrated prior to the actual operation, and the temperature of the distilled water must be known if a

TABLE 1.5: Corrections to be Added to the Apparent Weight of Water in Grams to Obtain Volume at 20°C of Glass Vessels Made of Soda-Lime Glass *

Temperature, °C	Tenths of degrees									
	0	1	2	3	4	5	6	7	8	9
15	0.207	0.208	0.210	0.211	0.212	0.213	0.215	0.216	0.217	0.219
16	.220	.221	.223	.224	.225	.227	.228	.230	.231	.232
17	.234	.235	.237	.238	.240	.241	.243	.244	.246	.247
18	.249	.250	.252	.253	.255	.257	.258	.260	.261	.263
19	.265	.266	.268	.270	.272	.273	.275	.277	.278	.280
20	.282	.284	.285	.287	.289	.291	.293	.294	.296	.298
21	.300	.302	.304	.306	.308	.310	.312	.314	.315	.317
22	.319	.321	.323	.325	.327	.329	.331	.333	.336	.338
23	.340	.342	.344	.346	.348	.350	.352	.354	.357	.359
24	.361	.363	.365	.368	.370	.372	.374	.376	.379	.381
25	.383	.386	.388	.390	.392	.395	.397	.399	.402	.404
26	.406	.409	.411	.414	.416	.418	.421	.423	.426	.428
27	.431	.433	.436	.438	.440	.443	.446	.448	.451	.453
28	.456	.458	.461	.463	.466	.469	.471	.474	.476	.479
29	.482	.484	.487	.490	.492	.495	.498	.501	.503	.506
30	.509	.511	.514	.517	.520	.522	.525	.528	.531	.534
31	.536	.539	.542	.545	.548	.551	.554	.556	.559	.562
32	.565									

* Values of 15–29.2°C. taken from "Standard Density and Volumetric Tables," *Natl. Bur. of Std., Circ.* 19. All values are based on an average cubical expansion of glass of 0.000025 ml/ml/°C. This figure corresponds closely to that of Kimble Standard Flint Glass for the temperature range shown in the table. The values in this table are reprinted through the courtesy of the Owens-Illinois Glass Co., Toledo, Ohio, from the booklet *The Care and Handling of Glass Volumetric Apparatus.*

maximum of accuracy is to be obtained. If a small article is being calibrated, the temperature may be recorded to the nearest degree, but if a large object (for example, a 1-liter flask) is under examination, the temperature should be known to the nearest tenth of a degree. Weighing by substitution is used in all gravimetric calibration procedures. The empty object to be calibrated or the container into which the water is to be drained is placed on one pan of the balance. A tare weight, heavier than the full container, is placed on the other pan. Weights are placed on the pan holding the empty vessel until equilibrium is established. After the container is filled with distilled water, weights are removed to secure balance equilibrium once again. The difference in the amount of weight before and after the filling operation is equal to the apparent weight of water at the recorded temperature. It should be noted at this point that most analytical balances have a maximum capacity of approximately 200 g. This automatically limits the size of the container that can be calibrated in the laboratory.

1. Calibration of Pipettes

A beaker is filled with distilled water, covered, and allowed to come to room temperature. The temperature of the water is then recorded. The pipette is filled and drained, and the operation is repeated two or three times until the temperature of the pipette is the same as that of the water. The pipette is now filled to approximately 20 mm above the etched calibration line. By using the index finger, the water level is carefully lowered to the zero line. The pipette is now held vertically and allowed to drain into a previously weighed weighing bottle. The water remaining in the tip is not removed unless the pipette is of the "blowout" type. Thirty to sixty seconds should be allowed for complete drainage, depending on the size of the pipette. The water and the weighing bottle are weighed and the volume of the pipette is calculated.

2. Calibration of Burettes

Burettes are calibrated to deliver a certain volume of liquid. Unlike a pipette, a burette has many capacity lines and, it is usual to check a number of these during the calibration process. As an example, a 50-ml burette is checked from 0 to 10 ml, 0 to 20 ml, to 30 ml, 40 ml, and 50 ml. The actual weighing operation is similar to that previously described.

Since burettes have a larger bore than pipettes, there is a greater possibility of error resulting from parallax. It is important, therefore, to read the burette properly during the calibration process and, of course, during the actual titration analysis of drugs. A dark piece of paper is placed behind the burette, and the volume is read to the bottom of the meniscus. The eye and the meniscus should be at the same level if the burette is to be read accurately. Figure 1.8 shows a typical titration assembly and the proper titration technique.

3. Calibration of Volumetric Flasks

Volumetric flasks are calibrated to contain the indicated volume. Flasks with volumes of 100 ml and less are weighed empty and then filled with distilled water; the true volume is then calculated. Larger flasks must be weighed on special balances.

B. CARE OF VOLUMETRIC GLASSWARE

Pipettes, burettes, and volumetric flasks must be scrupulously clean to permit the accuracy required in pharmaceutical analysis. Such glassware is clean if the inside surface is uniformly wetted with distilled water. Droplets of water along the drained surfaces indicate that the apparatus is contaminated and must not be used in any analytical operation.

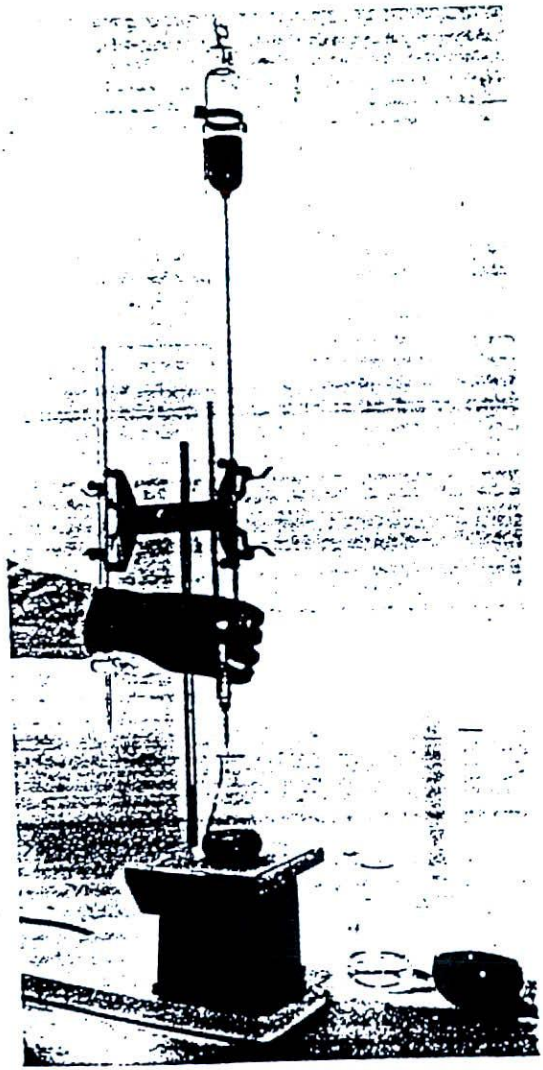

FIGURE 1.8: Microburettes and magnetic stirrers provide the accuracy and convenience required in many analytical operations.

Strong alkaline solutions will attack glass and should not be used in any cleaning operations. However, dilute detergent solutions (to approximately 2%) and elbow grease are still the method of choice for cleaning most of the glassware used in the laboratory. Contact between the glass and the solution should not be prolonged, and the apparatus must be well-rinsed with distilled water after being cleaned.

Grease may be removed by soaking the apparatus in gasoline or acetone. Sulfuric acid-bichromate mixtures may be used to remove some contaminants. This mixture may be made by dissolving 60 g of potassium bichromate in 30 ml of water with the aid of heat, cooling, and then slowly adding concentrated sulfuric acid to make 1 liter of solution. (In addition to cleaning glassware, this solution has been used by some students to burn holes in clothing, remove the surfaces of working areas, and study the effect of the mixture on exposed body parts.) This lethal mixture should never be handled carelessly and must not be poured at eye level unless the analyst is wearing safety goggles. The apparatus should be rinsed at least six times with distilled water, after being cleaned with this mixture.

Stopcock grease is one of the main laboratory contaminants. Burette tips are often clogged with such grease, which may be removed by heating the tip gently and then soaking in a cleaning solution. Silicone grease is particularly troublesome and should be used sparingly. Warm decahydronaphthalene (decalin) will remove silicone grease. The apparatus should be soaked for 2 hr and then rinsed with acetone.

1.6 THE ART AND SCIENCE OF PHARMACEUTICAL ANALYSIS

The analysis of those chemicals we call drugs is based on sound scientific principles. Chemistry and mathematics are subtly combined in such classical techniques as gravimetry and titrimetry and in the more modern approaches to precise measurement, as exemplified by absorptimetry and radioactimetry. However, there is more to the analysis of drugs than that implied by this statement. Even among the scientific elite, it is accepted that certain highly trained scientists have little mastery over the most fundamental laboratory procedures. It is fortunate, however, that both the "book" man and the "lab" man have a place in the sun. The lab man knows the tools and techniques of his profession and, more important, is able to exercise judgment and to transcribe that indefinable state of mind into action in the laboratory.

Pharmacopeias and/or drug-control legislation permit a manufacturer to market a product that contains slightly less or slightly more than that claimed on the label. The maximum deviation from label claim is of the order of 10% and, in many instances, the pharmacopeias reduce this permitted deviation to approximately 5%. In addition, pharmacopeias provide the analyst with

monographs that deal with pure drugs or chemicals, and these may allow for no more than 1 to 1.5% impurity. With respect to the dosage form, these deviations are permitted for three reasons. First, it is understood that no drug can be put into a dosage form without some compounding error. Second, some drugs are unstable and gradually deteriorate on standing. The pharmacopeias recognize this and sum the effect into the total allowable deviation from label claim. Third, no method of analysis is so precise that it provides the analyst with recovery values free of error. Again, the pharmacopeias recognize this and allow for such error in the permitted deviations. The analyst has no control over the first two factors, but he can ensure that the error inherent in his analytical procedure is as small as possible. He does this by performing all operations carefully and quantitatively, by insisting on a maximum of cleanliness in the laboratory, and by paying particular attention to the theoretical implications of each step in the analytical procedure.

The art of pharmaceutical analysis is mastered in the laboratory and, in this respect, experience is the best teacher. Some laboratory practices are outlined below but with the understanding that there are several approaches to the same analytical operation. Those techniques described in this section have stood the test of time and are generally recommended to the fledgling analyst.

A. CHOOSING THE TOOLS

A method of analysis rarely specifies the type of container, flask, separatory funnel, pipette, or burette that is to be used in a particular operation. The analyst must, therefore, use common sense and choose the right tools for the operation at hand. For example, a 100-ml graduated cylinder would not be used to measure 10 ml of solution. Similarly, a microtitration would be carried out by using a microburette, and the flask in which the solution and titrant are combined should bear a reasonable relationship to the total volume of liquid. It is obviously wrong to use a 250-ml Erlenmeyer flask if the total volume does not exceed 50 ml. In such titrations, a 125-ml Erlenmeyer flask is the container of choice. There are, of course, many examples that might be cited here but this would serve no useful purpose. The analyst must study the method and, on the basis of experience and/or common sense, choose the tools that provide a maximum of efficiency and a minimum of error.

B. IDENTIFICATION OF CONTAINERS

A method of analysis is not complete unless it has been carried out at least two, and preferably three, times. These separate determinations are generally performed at the same time, and it is very important to mark carefully each container that is used in completing the operation. By neglecting this seemingly insignificant operation, the student (and, often, the practicing analyst) can lose a whole afternoon's work. Too often, the

student's lament is that he cannot remember which sample is in which beaker.

Laboratory supply companies sell a wide variety of pens, pencils, marking inks, and marking instruments. Each item has a particular use, and the student is referred to the various catalogs for specific descriptions. In general, the marking inks and marking instruments permanently mark glassware and porcelain, whereas the various wax pencils and china-marking pens contain materials that can be removed with soap and water or by soaking in various organic solvents.

C. FILTRATION

Solids suspended in liquids are often encountered during the analysis of pharmaceuticals. In certain instances, the solid must be quantitatively recovered for further processing; in other procedures, the suspended solid is removed and discarded before the analysis is continued. Both operations are carried out by passing the liquid through a filter to remove the solids.

The simplest approach to the separation of suspended solids from liquids is to filter the fluid through a small pledget of cotton wool packed into the neck of a filtering funnel. The cotton wool effectively removes coarse particles from the liquid but may permit the passage of finer materials into the filtrate. The procedure is not generally recommended, but it may be used in those situations where small amounts of foreign material have little effect on the results of the analysis.

The classical approach to filtration depends on the use of filter paper mounted inside a filtering funnel. The many types of filter paper differ in size, porosity, wet strength, and ash content. Each type has a particular use; and it is beyond the scope of this book to describe in detail the characteristics and purposes of the various grades of paper. However, the student should understand that some grades of paper cannot be used for certain specific operations. For example, Whatman No. 1 filter paper (or its equivalent) retains precipitates of average fineness, allows fluid to pass through fairly rapidly, but cannot be used for quantitative purposes. If a solid is to be recovered by filtration, ignited, and analyzed gravimetrically, an acid-washed (to remove inorganic material) low-ash paper must be used. Examples of such paper are Whatman No. 40, No. 41, and No. 42. The first filter paper is recommended for the recovery of fine crystals, the second for coarse and gelatinous precipitates, and the third for very fine crystals. Filter papers similar to those described above are also marketed under the Schleicher and Schuell (S & S) and Munktells labels. At most, an 11-cm circle of quantitative filter paper will yield approximately 0.15 mg of ash.

The diameter of a circle of filter paper may be as little as 4.25 cm and as much as 50 cm. The size of the filtering funnel governs the size of paper that is to be used in the operation. The paper should be folded into quarters, seated carefully into the funnel, and wetted with solvent until a tight seal is obtained between the paper and the glass. The top of the paper should not

extend above the edge of the funnel but rather should come to within 2 cm of the rim. (Some analysts suggest that the filter paper should not form a tight seal with the funnel. If there is a space between the paper and the glass, filtration is faster. This space is produced by folding the paper symmetrically and then, on the second fold, not quite symmetrically. A filter paper folded in this manner will fit a 60° funnel only at the top. However, it is important

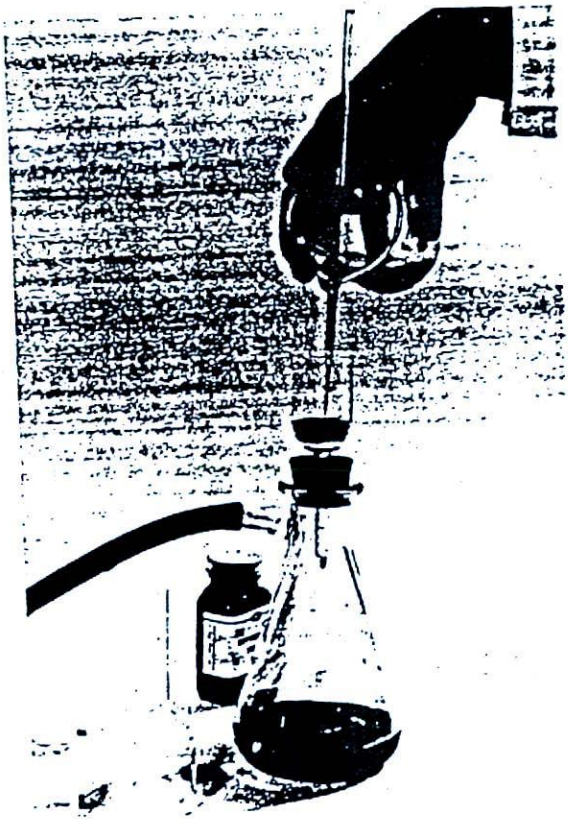

FIGURE 1.9: Use of a Glass Filtering Crucible.

that a tight seal be established between the paper and the glass near the rim of the funnel.)

Filtration generally is a slow procedure, but it can be speeded up by the use of a filter flask and suction. These flasks are usually equipped with Gooch or glass filtering crucibles with sintered-glass bottoms. A typical filtration setup is shown in Fig. 1.9. The glass crucibles illustrated in this figure have sintered-glass bottoms whose porosities are designated as fine, medium, or coarse.

They are available in various sizes and shapes and have been readily accepted by the practicing analyst. Some laboratories, however, still make use of the Gooch crucible. This crucible has a bottom pierced with small holes. These holes are covered with a small disc of filter paper or with an asbestos mat formed by pouring a slurry of asbestos fibers through the Gooch. Few laboratories use this last technique.

D. IGNITION OF PRECIPITATES

Some precipitates may be collected in sintered-glass crucibles and heated to as high as 500°C in a muffle furnace. This temperature must be approached gradually and, if an open flame is used, the sintered-glass crucible should be placed in an ordinary porcelain crucible before heat is applied. Sintered-glass crucibles are susceptible to sudden changes in temperature and cannot be heated over a direct flame. The sintered surfaces are easily ruined by carbon deposits, and the desired product may be converted into some unwanted substance by the gases from the flame penetrating through the porous bottom. In the hands of expert analysts, these crucibles have a definite use, but the student rarely uses them for the ignition of precipitates.

If a precipitate must be ignited at a temperature in excess of 500°C, it is collected on ashless filter paper, transferred to a porcelain crucible, and heated to the required temperature. The top of the filter paper is first pulled away from the funnel surface and carefully folded over to enclose completely the precipitate. The filter paper and the precipitate are then transferred to a porcelain crucible and dried in an oven or over a small flame. The crucible is then mounted in a slanted position on a clay or wire triangle. A crucible cover is positioned, at an angle, on top of the crucible and heat is applied The charring process must be gradual and the paper should not ignite. If the paper catches fire, the crucible must be covered immediately to extinguish the flame. Once charring is complete, the crucible is heated until it becomes a full red and no dark-colored material is left on the surfaces. The crucible is then placed upright, the cover is set slightly to one side, and the heating is continued for as long as is necessary.

Porcelain crucibles may be heated to 750°C by using a Bunsen burner, to 950°C by using a Tirrell burner, and to 1000°C by using a Meker or Fischer burner. The crucible will glow at 500°C, will turn red at 600°C, and will appear orange at approximately 1000°C.

E. DRYING THE SAMPLE

Most solids tend to absorb moisture when exposed to the atmosphere. This moisture must be removed before the sample is weighed or treated in some other manner. There are exceptions to this rule, and it is necessary, therefore, to consider the natural state of the substance being analyzed.

For example, ephedrine may contain as much as 6% water and is analyzed without being subjected to a drying process. Some drugs may contain water of crystallization and, since this forms part of the molecule, the analysis is carried out with this in mind. If moisture is adsorbed at some stage in the analytical process, it must be removed. If it is present before the analysis begins, the analyst must exercise common sense and decide if it should be removed. If moisture is a natural contaminant, the drug should be analyzed without prior treatment. However, if the drug is being used as a primary standard in some analytical process, the moisture must be removed before the substance is weighed.

Many chemicals melt at temperatures in excess of 100°C and may be dried in a laboratory oven set at 105°C, or higher, if the circumstances so warrant. Exposure times vary with the drug and are usually specified in the analytical procedure. Many drugs tend to darken when exposed to oven temperatures, but these may be dried at lower temperatures if a vacuum oven is available. These ovens are inconvenient to use and require auxiliary equipment to produce the vacuum, but they will dry the sample quickly and effectively.

As soon as the sample is dry, it is removed from the oven, cooled to room temperature, and placed in a desiccator. This glass vessel serves as a storage place for objects and chemicals that must be kept in an atmosphere relatively free of moisture. A drying agent or desiccant is placed in the bottom of the vessel, and it is this substance that determines the amount of water vapor in the atmosphere. Common desiccants, in order of increasing effectiveness, are calcium chloride, calcium oxide, sulfuric acid, calcium sulfate, magnesium perchlorate, and phosphorus pentoxide. Desiccants such as sulfuric acid and phosphorus pentoxide are usually transferred to a shallow dish of suitable diameter and then placed in the bottom of the desiccator. This dish may be easily removed when the drying agent is exhausted. Water combines violently with both of these drying agents, and the containers should be handled carefully during the cleaning operation.

Phosphorus pentoxide, when in equilibrium with the air in the desiccator, will effectively remove most of the water vapor. As it absorbs moisture, it tends to form a surface glaze. This glaze must be removed if the bulk of the material is to combine effectively with water vapor within the desiccator. Air, in equilibrium with calcium chloride, will contain approximately 0.5 mg of water per liter. It is, therefore, not as effective as phosphorus pentoxide, but it is inexpensive, has a high capacity (that is, it can take up much water per unit weight of drying agent), and is suitable for most drying operations. Colored calcium sulfate, marketed as "Drierite," is also widely used as a desiccant. It derives its blue color from a cobalt salt mixed with calcium sulfate and turns pink when exhausted, and may be regenerated by heating it in an oven.

Hot objects are not cooled in a closed desiccator. If a hot object is so cooled, a partial vacuum is created, and, on opening the desiccator, air rushes in and

tends to blow material out of the containers resting on the porcelain plate. The hot object may be placed in the desiccator and cooled but the top should be only partially covered during this operation. Once the temperature of the object nears that of the room, the desiccator can be closed and the cooling continued for at least 30 min before the sample is weighed.

A standard desiccator includes the vessel proper, a porcelain plate, and a cover. The rims of the vessel and the cover have ground-glass surfaces, which should be lightly coated with a lubricant when the desiccator is first put into use. Some desiccator covers are equipped with valves, which permit partial evacuation of the air from the chamber. However, desiccators are rarely used in this manner; if they are, specially constructed metal containers are preferable to the standard glass vessels used in most laboratories.

F. RECORDING RESULTS

The student in the laboratory is not bound to the laws that govern the analysis and distribution of pharmaceuticals. On the other hand, the practicing analyst is, and it is for this reason that he pays particular attention to the recording of the analytical results. It is the analyst who gives his stamp of approval to a particular pharmaceutical and, if circumstances so warrant, he must be able to recall all data pertinent to that product. Individuals differ in their ability to remember facts and figures, but no analyst is excused from the task of keeping a neat and detailed laboratory record book. Although the student gives no stamp of approval to any product, the instructor expects him to mimic the practicing analyst and maintain proper records as he proceeds, step by step, through the analytical procedure.

The laboratory record book should be bound, and all results must be recorded in ink as they are accumulated. The pages should be numbered and each page, as it is used, is dated and signed by the analyst. Results and procedural details should be entered in the book so that a second analyst can easily repeat the work and check all calculations. Each instructor will recommend to the student some particular order of presentation of data and analytical observations and, at the same time, will indicate the manner in which the results are to be reported to him for grading purposes. The student often considers the laboratory report that is handed in to the instructor as the most important aspect of record keeping. Although this may be true with respect to the final grade in the course, the results reported therein are drawn from the laboratory record book, and it is there that the following information should be found: name of the drug being analyzed, description of dosage form, lot or identification number, property determined, sample size, procedure used—either in detail or by reference to some readily available literature source, all results, and the date on which the analysis was carried out.

The proper recording of results cannot be over-emphasized. A good record

book lends itself to the easy checking of calculations and this, in itself, has stopped many students from reporting foolish results. Some students do not appreciate the necessity of recording results directly into their record book. Results recorded on bits of paper tend to get lost (or are deliberately picked up by laboratory instructors) or make little sense to the analyst several days after the analysis has been performed. The teacher has little sympathy for those students that record their results in such a manner.

REFERENCES

1. "Quality Without Qualification," *Tile and Till*, 49, 62 (1963).
2. Brodie, D. C., *Am. J. Hosp. Pharm.*, 17, 407 (1960).
3. Cowles, R. S., *Bio-Medical Purtiew*, 3, 51 (1963).
4. *Lancet*, 1963-II, 283.
5. Frimpter, G. W., A. E. Timpanelli, W. J. Eisenmenger, H. S. Stein, and L. I. Ehrlich, *J. Am. Med. Assoc.*, 184, 111 (1964).
6. Dunnett, C. W., and R. Crisafio, *J. Pharm. Pharmacol.*, 7, 314 (1955).
7. Moskalyk, R. E., L. G. Chatten, C. E. Cox, and M. Pernarowski, *J. Pharm. Sci.*, 50, 651 (1961).
8. Breunig, H. L., and E. P. King, *J. Pharm. Sci.*, 51, 1187 (1962).
9. United States Department of Commerce, *Natl. Bur. Std., Circ.* 547, Sec. 1, August 20, 1954.