within (±15%) of the average value obtained for the replicate measurements of the *Standard solution*. The average value of the replicate measurements of *Spiked sample solution 2* must provide a signal intensity or value less than that of the *Standard solution*. [NOTE—Correct the values obtained for each of the spiked solutions using the *Unspiked sample solution*.]

Precision for Instrumental Methods (Repeatability)

[NOTE—Non-instrumental precision is demonstrated by meeting the *Detectability* requirement above.]

Sample solutions: Six independent samples of the material under test, spiked with appropriate reference materials for the *Target Elements* at the *Target Concentration*.

Acceptance criteria

Relative standard deviation: NMT 20% for each *Target Element*.

Specificity

The procedure must be able to unequivocally assess (see *Validation of Compendial Procedures* ⟨1225⟩) each *Target Element* in the presence of components that may be expected to be present, including other *Target Elements*, and matrix components.

QUANTITATIVE PROCEDURES

The following section defines the validation parameters for the acceptability of alternative quantitative procedures. Meeting these requirements must be demonstrated experimentally, using an appropriate system suitability procedure and reference materials. Meeting these requirements demonstrates that the procedure is equivalent to the compendial procedure for the purpose of quantifying the *Target Elements*.

Accuracy

Standard solutions: Prepare solutions containing the *Target Elements* at concentrations ranging from 50% to 150% of *J*, using appropriate reference materials.

Test samples: Prepare samples of the material under test spiked with appropriate reference materials before any sample preparation steps (digestion or solubilization) at concentrations ranging from 50% to 150% of *J* for each *Target Element*.

Acceptance criteria

Spike recovery: 70%–150% for the mean of three replicate preparations at each concentration

Precision

REPEATABILITY

Test samples: Six independent samples of material under test (taken from the same lot) spiked with appropriate reference materials for the *Target Element(s)* at the indicated level.

Acceptance criteria

Relative standard deviation: NMT 20% for each *Target Element*

RUGGEDNESS

Perform the *Repeatability* analysis over three independent events using the following events or combinations thereof:

1. on different days, or
2. with different instrumentation, or
3. with different analysts.

Acceptance criteria

Relative standard deviation: NMT 25% for each *Target Element*

Specificity

The procedure must be able to unequivocally assess (see *Validation of Compendial Procedures* ⟨1225⟩) each *Target Element* in the presence of components that may be expected to be present, including other *Target Elements*, and matrix components.

Limit of Quantitation, Range, and Linearity

Demonstrated by meeting the *Accuracy* requirement.
■2S (USP35)

Physical Tests and Determinations

⟨616⟩ BULK DENSITY AND TAPPED DENSITY OF POWDERS

Change to read:

BULK DENSITY

This general chapter has been harmonized with the corresponding texts of the *European Pharmacopoeia* and/or the *Japanese Pharmacopoeia*. ✦The portion that is not harmonized is marked with symbols (✦✦) to specify this fact.✦

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of the interparticulate void volume. Hence, the bulk density depends on both the density of powder particles and the spatial arrangement of particles in the powder bed. The bulk density is expressed in grams per mL (g/mL) although the international unit is kilograms per cubic meter (1 g/mL = 1000 kg/m^3) because the measurements are made using cylinders. It may also be expressed in grams per cubic centimeter (g/cm^3). The bulking properties of a powder are dependent upon the preparation, treatment, and storage of the sample, i.e., how it was handled. The particles can be packed to have a range of bulk densities; however, the slightest disturbance of the powder bed may result in a changed bulk density. Thus, the bulk density of a powder is often very difficult to measure with good reproducibility and, in reporting the results, it is essential to specify how the determination was made. The bulk density of a powder is determined by measuring the volume of a known weight of powder sample, that may have been passed through a ■sieve■2S (USP35), into a graduated cylinder (*Method I*), or by measuring the mass of a known volume of powder that has been passed through a volumeter into a cup (*Method II*) or a measuring vessel (*Method III*).

Method I and *Method III* are favored.

Method I—Measurement in a Graduated Cylinder

Procedure—Pass a quantity of material sufficient to complete the test through a sieve with apertures greater than or equal to 1.0 mm, if necessary, to break up agglomerates that may have formed during storage; this must be done gently to avoid changing the nature of the material. Into a dry graduated 250-mL cylinder (readable to 2 mL) introduce, without compacting, approximately 100 g of test sample, *M*, weighed with 0.1% accuracy. Carefully level the powder without compacting, if necessary, and read the unsettled apparent volume (V_0) to the nearest graduated unit. Calculate the bulk density in g/mL by the formula m/V_0. Generally, replicate determinations are desirable for the determination of this property. If the powder density is too low or too high, such that the test sample has an untapped apparent volume of either more than 250 mL or less than 150 mL, it is not possible to use 100 g of powder sample. Therefore, a different amount of powder has to be selected as the test sample, such that its untapped apparent volume is 150–250 mL (apparent volume greater than or equal to 60% of the total volume of the cylinder); the weight of the test sample is specified in the expression of results. For test samples having an apparent volume between 50 mL and 100 mL, a 100-mL cylinder readable to 1 mL can be used; the volume of the cylinder is specified in the expression of results.

Method II—Measurement in a Volumeter

Apparatus—The apparatus (*Figure 1*) consists of a top funnel fitted with a 1.0-mm ■sieve■2S *(USP35)*.[1] The funnel is mounted over a baffle box containing four glass baffle plates over which the powder slides and bounces as it passes. At the bottom of the baffle box is a funnel that collects the powder and allows it to pour into a cup of specified capacity mounted directly below it. The cup may be cylindrical (25.00 ± 0.05 mL volume with an inside diameter of 30.00 ± 2.00 mm) or ■cubical■2S *(USP35)* (16.39 ± 0.2 mL volume with inside dimensions of ■25.400■2S *(USP35)* ± 0.076 mm).

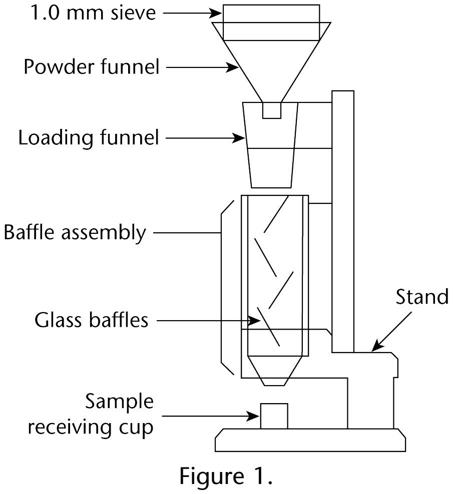

Figure 1.

Procedure—Allow an excess of powder to flow through the apparatus into the sample receiving cup until it overflows, using a minimum of 25 cm³ of powder with the square cup and 35 cm³ of powder with the cylindrical cup. Carefully scrape excess powder from the top of the cup by smoothly moving the edge of the blade of a spatula perpendicular to and in contact with the top surface of the cup, taking care to keep the spatula perpendicular to prevent packing or removal of powder from the cup. Remove any material from the sides of the cup, and determine the weight, *M*, of the powder to the nearest 0.1%. Calculate the bulk density, in g/mL, by the formula:

$$(M)/(V_0)$$

in which V_0 is the volume, in mL, of the cup. Record the average of three determinations using three different powder samples.

Method III—Measurement in a Vessel

Apparatus—The apparatus consists of a 100-mL cylindrical vessel of stainless steel with dimensions as specified in *Figure 2*.

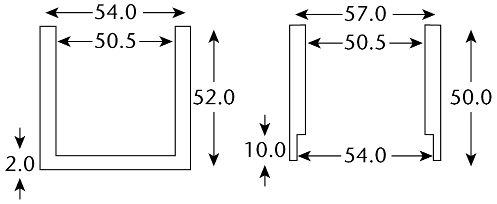

Figure 2.

Procedure—Pass a quantity of powder sufficient to complete the test through a 1.0-mm sieve, if necessary, to break up agglomerates that may have formed during storage, and allow the obtained sample to flow freely into the measuring vessel until it overflows. Carefully scrape the excess powder from the top of the vessel as described for *Method II*. Determine the weight (M_0) of the powder to the nearest 0.1% by subtraction of the previously determined mass of the empty measuring vessel. Calculate the bulk density (g/mL) by the formula $M_0/100$, and record the average of three determinations using three different powder samples.

Change to read:

TAPPED DENSITY

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample. Tapped density is obtained by mechanically tapping a graduated measuring cylinder or vessel containing a powder sample. After observing the initial powder volume or weight, the measuring cylinder or vessel is mechanically tapped, and volume or weight readings are taken until little further volume or weight change is observed. The mechanical tapping is achieved by raising the cylinder or vessel and allowing it to drop under its own weight a specified distance by either of three methods as described below. Devices that rotate the cylinder or vessel during tapping may be preferred to minimize any possible separation of the mass during tapping down.

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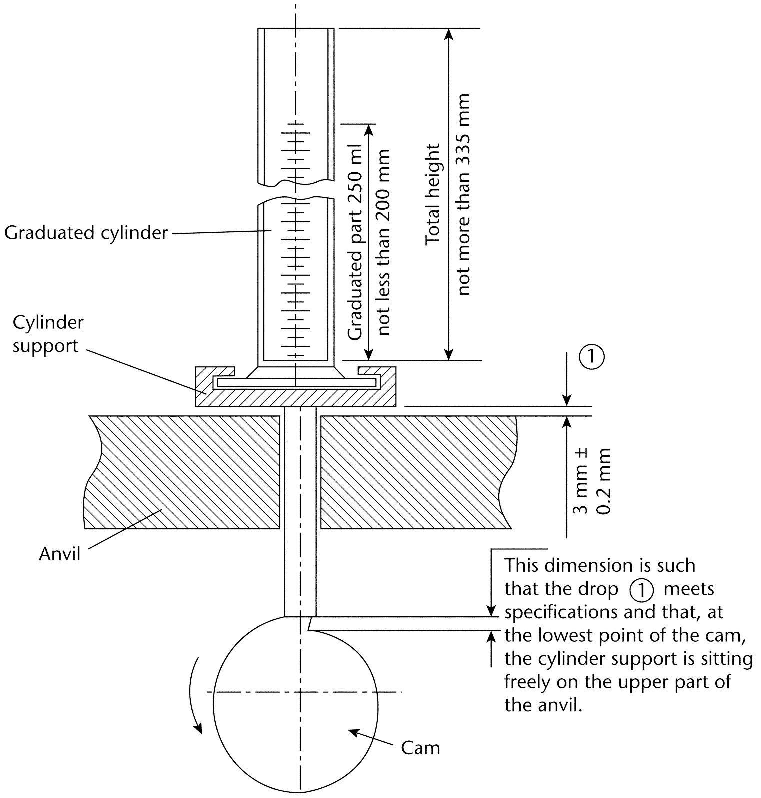
[1] The apparatus (the Scott Volumeter) conforms to the dimensions in ASTM 329 90.



This dimension is such that the drop ① meets specifications and that, at the lowest point of the cam, the cylinder support is sitting freely on the upper part of the anvil.

Figure 3.

Method I

Apparatus—The apparatus (*Figure 3*) consists of the following:

- A 250-mL graduated cylinder (readable to 2 mL with a mass of 220 ± 44 g)
- A settling apparatus capable of producing, in 1 min, either nominally 250 ± 15 taps from a height of 3 ± 0.2 mm, or nominally 300 ± 15 taps from a height of 14 ± 2 mm. The support for the graduated cylinder, with its holder, has a mass of 450 ± 10 g.

Procedure—Proceed as described above for the determination of the bulk volume (V_0). Secure the cylinder in the holder. Carry out 10, 500, and 1250 taps on the same powder sample and read the corresponding volumes V_{10}, V_{500}, and V_{1250} to the nearest graduated unit. If the difference between V_{500} and V_{1250} is less than ■or equal to■2S (USP35) 2 mL, V_{1250} is the tapped volume. If the difference between V_{500} and V_{1250} exceeds 2 mL, repeat in increments such as 1250 taps, until the difference between succeeding measurements is less than ■or equal to■2S (USP35) 2 mL. Fewer taps may be appropriate for some powders, when validated. Calculate the tapped density (g/mL) using the formula m/V_F, in which V_F is the final tapped volume. Generally, replicate determinations are desirable for the determination of this property. Specify the drop height with the results. If it is not possible to use a 100-g test sample, use a reduced amount and a suitable 100-mL graduated cylinder (readable to 1 mL) weighing 130 ± 16 g and mounted on a holder weighing 240 ± 12 g. The modified test conditions are specified in the expression of the results.

Method II

Apparatus and **Procedure**—Proceed as directed under *Method I* except that the mechanical tester provides a fixed drop of 3 ± 0.2 mm at a nominal rate of 250 taps per min.

Method III

Apparatus and **Procedure**—Proceed as directed in *Method III—Measurement in a Vessel* for measuring bulk density using the measuring vessel equipped with the cap shown in *Figure 2*. The measuring vessel with the cap is lifted 50–60 times per min by the use of a suitable tapped density tester. Carry out 200 taps, remove the cap, and carefully scrape excess powder from the top of the measuring vessel as described in *Method III—Measurement in a Vessel* for measuring the bulk density. Repeat the procedure using 400 taps. If the difference between the two masses obtained after 200 and 400 taps exceeds 2%, carry out a test using 200 additional taps until the difference between succeeding measurements is less than 2%. Calculate the tapped density (g/mL) using the formula $M_F/100$, where M_F is the mass of powder in the measuring vessel. Record the average of three determinations using three different powder samples. The test conditions including tapping height are specified in the expression of the results.

MEASURES OF POWDER COMPRESSIBILITY

Because the interparticulate interactions influencing the bulking properties of a powder are also the interactions that interfere with powder flow, a comparison of the bulk and tapped densities can give a measure of the relative importance of these interactions in a given powder. Such a comparison is often used as an index of the ability of the powder to flow, for example the *Compressibility Index* or the *Hausner Ratio* as described below.

The *Compressibility Index* and *Hausner Ratio* are measures of the propensity of a powder to be compressed as described above. As such, they are measures of the powder's ability to settle, and they permit an assessment of the relative importance of interparticulate interactions. In a free-flowing powder, such interactions are less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, there are frequently greater interparticle interactions, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the *Compressibility Index* and the *Hausner Ratio*.

Compressibility Index—Calculate by the formula:

$$100(V_0 - V_F)/V_0$$

V_0 = unsettled apparent volume
V_F = final tapped volume

Hausner Ratio—

$$V_0/V_F$$

Depending on the material, the compressibility index can be determined using V_{10} instead of V_0. [NOTE—If V_{10} is used, it will be clearly stated in the results.]

Change to read:

■⟨696⟩ CHARACTERIZATION OF CRYSTALLINE SOLIDS BY MICROCALORIMETRY AND SOLUTION CALORIMETRY

For the purpose of this chapter, crystalline material, partially crystalline material, and amorphous material are considered as solids.

INTRODUCTION—THE CONCEPT OF CRYSTALLINITY

The perfectly ordered crystal lattice with every molecule in its expected lattice position is an ideal that is seldom, if ever, achieved. The other extreme is the amorphous state, in which a solid contains the maximum possible density of imperfections (defects of various dimensionalities), such that all long-range order is lost while only the short-range order, imposed by its nearest neighbors, remains. Real crystals lie somewhere between these two extremes. A crystal's position on a scale bounded by these two extremes is termed *crystallinity*.

All real crystals, even in the pure state, possess some lattice imperfections or defects, which increase both the energy (enthalpy under conditions of constant atmospheric pressure) and the disorder (expressed as the entropy) of the crystal lattice. A crystal with a relatively low density of imperfections is said to be highly crystalline and to possess a high crystallinity. By contrast, a particle with a relatively high density of imperfections is said to be partially amorphous and to possess a low crystallinity. In ideal terms, a totally amorphous particle corresponds to zero crystallinity. Amorphous particles may contain somewhat ordered domains that can act as nuclei for crystallization; such so-called amorphous particles are said to possess a low-level, but finite, crystallinity.

The ability to detect and quantify the amount of amorphous material within a highly crystalline substance is of great importance during the development and subsequent manufacture of a pharmaceutical preparation.

In reality, a powder probably contains particles with different degrees of crystallinity, just as it may contain particles with varying sizes and shapes. The lower the crystallinity of a solid, the greater its enthalpy and entropy. The increase in enthalpy is never totally compensated by the increase in entropy; therefore, the Gibbs free energy, which reflects the balance between them, actually increases. Hence, the lower the crystallinity of a material (powder), and consequently the greater its amorphous character, the greater its apparent intrinsic solubility and dissolution rate, but the lower its thermodynamic stability. Because of the great relevance of these properties, crystallinity is also an important property and requires measurement by a suitable method.

In the following chapter, the crystallinity or the content of amorphous parts of a powder are measured by calorimetric methods such as microcalorimetry or solution calorimetry, although other methods could be used (e.g., see general chapter *Characterization of Crystalline and Partially Crystalline Solids by X-ray Powder Diffraction (XRPD)* ⟨941⟩).

Many substances are capable of crystallizing in more than one type of crystal lattice, which is known as polymorphism. If water or a solvent is incorporated in the crystal lattice, the crystals are termed hydrates or solvates. Because of the different crystal packing, and/or molecular conformation and lattice energy, they usually exhibit different physical properties. For simplicity, calorimetry measurements for degree of crystallinity determination discussed here assume only one solid crystalline form present in the material of interest. The theory and experimental technique can be easily expanded to polymorphic systems with proper consideration of the enthalpy differences among the polymorphs.

METHOD 1—MICROCALORIMETRY (DETERMINATION OF AMORPHOUS CONTENT)

Most chemical, physical, and biological processes are associated with the exchange of heat. Microcalorimetry is a highly sensitive technique to monitor and quantify both exothermic (heat producing) and endothermic (heat absorbing) changes associated with those processes. The technique allows the determination of the rate and extent of chemical reactions, changes of phase, or changes of structure.

Thermal events producing only a fraction of a microwatt can be observed using microcalorimetry. This means that temperature differences less than 10^{-6} K must be detectable. Microcalorimetry typically uses the heat flow (heat leakage) principle, where, in a thermally defined vessel, the heat produced (or absorbed) flows away from (or into) the vessel in an effort to re-establish thermal equilibrium with its surroundings. Exceptional thermal stability with its surrounding has to be achieved either by a heat sink or an electronically regulated surrounding.

Heat energy from an active sample in the reaction vessel is channeled typically through Peltier elements; they act as thermoelectric generators using the Seebeck effect. The heat energy is converted into a voltage signal proportional to the heat flow.

Results are typically presented as a measure of the thermal energy produced per unit of time (Watt) as a function of time.

Apparatus

Microcalorimeters are typically designed as twin systems with a measuring vessel and a reference vessel. Vessels are typically made of glass or stainless steel. For certain applications, specially designed vessels that allow the addition of a gas, a liquid, or a solid material may be used.