Chapter 2

Preparing Soils for Mineralogical Analyses

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Sample preparation is an important aspect of soil mineralogical analysis. The use of pre-treatments is often necessary to facilitate sample dispersion and/or to concentrate a particular size fraction for subsequent analyses. However, pretreatments may alter, or even destroy, certain fractions of the soil (Kunze and Dixon, 1986). The pretreatments described in the following sections of this chapter are designed to have a minimum effect on constituents other than those being eliminated. In spite of this, the analyst must still be careful to perform only those pretreatments that are essential to accomplish the study objectives. Additionally, the analyst should have a clear understanding of the possible consequences for data interpretation. The reagents used for the various pretreatments do not need to be prepared with a high degree of precision. For example, the reagents may be mixed in beakers rather than in a volumetric flask.

SOIL PREPARATION

As discussed in Chapter 1 (Soukup et al., 2008, this volume), special field sampling and preservation techniques are required for organic soils, permafrost-affected soils, and Vertisols (Soil Survey Staff, 1996; Schoeneberger et al., 2002). To preserve organic soils, Blevins et al. (1968) recommended freezing blocks of soil in the field with liquid N₂ and transporting them to the laboratory in a frozen state. Other soils, including those affected by salts, reducing conditions, or high water contents, may also require special sampling, transportation, and preparation techniques before mineralogical analysis. Once collected, it is critical that such samples be transported and stored under conditions similar to those existing at the field site because many of the minerals in such soils may hydrate, dehydrate, oxidize, or dissolve during transport and storage. We strongly recommend that when working with potentially “problematic” soils researchers consult the literature, local NRCS personnel and other investigators regarding sampling, transport, and preparation techniques that have been employed previously on similar soils. The relative merits of each technique must be evaluated on a soil by soil basis to determine the most appropriate method(s). We also recommend that when there is the potential for mineralogical and other changes, samples should be analyzed as quickly as possible after sampling.

The amount of soil required for mineralogical analyses will vary depending on the study objectives and the sample characteristics. Generally, a sufficient amount of sample...
should be utilized to yield approximately 5 to 10 g of clay (<2-µm fraction) following the necessary pretreatments and subsequent fractionation procedures.

**REMOVAL OF SOLUBLE SALTS, GYPSUM, AND CARBONATES**

The presence of soluble salts and/or gypsum in soil samples may make it difficult or impossible to disperse the soil samples before fractionation procedures, because of the flocculating action of the salts (Kunze and Dixon, 1986). Soluble salts may also prevent saturation of the exchange complex with a specific cation for determination of cation exchange capacity. As will be discussed below, it is possible to first identify and quantify the soluble salts and gypsum present in the sample using X-ray diffraction (XRD) in combination with other mineralogical analyses. This is especially important as the soluble salt or gypsum content of some soils can be >80% (Buck et al., 2006a, 2006b). After this has been achieved, removing the soluble salts and gypsum is recommended because it simplifies other mineralogical analyses (such as XRD and differential thermal analyses [DTA]) (Kunze and Dixon, 1986). If done in this order, then identification and quantification of all soil constituents is possible.

Carbonates may also make it difficult or impossible to fractionate the sample and effectively separate the silt and clay fractions. Moreover, the presence of carbonates may result in poor X-ray diffractograms, because the degree of orientation of clay-sized particles (i.e., <2 µm) is decreased in the presence of finely divided carbonates (Kunze and Dixon, 1986). As with soluble salts and gypsum, carbonates may be identified and quantified using a variety of mineralogical and chemical analyses. This is important because the carbonate contents in some calcareous soil may comprise more than 80% of the soil mass (Loeppert and Suarez, 1996).

**Test for Presence of Soluble Salts and Gypsum**

**Soluble Salts**

The presence of soluble salts in a sample is indicated by elevated electrical conductivity (EC) measurements in the soil solution. Pure water is a poor conductor of electricity, but conductivity increases as more salt is dissolved in the water. Thus, the EC of the soil solution provides an indirect measurement of the salt content. As an initial test for soluble salts, a soil sample is saturated with distilled water and mixed into a paste. The EC of the water-saturated soil paste may be measured. Alternatively, the water may be extracted from the soil paste, and the EC of the saturated paste extract measured (Soil Survey Staff, 1996). It is difficult to suggest a lower limit of EC below which removal is not necessary, because of the variability in the properties of the soluble salts occurring in soils (Kunze and Dixon, 1986). However, if the EC of the saturated paste or saturated paste extract is in excess of 2 to 4 dS m⁻¹, then treatment to remove excess soluble salts is recommended.

**Gypsum**

The presence of gypsum in a soil sample can be determined qualitatively using the following procedure (Soil Survey Staff, 1996).

**Reagents**

- Acetone, reagent grade
Preparing Soils

Equipment and Materials
- 250-mL bottle or Erlenmeyer flask
- Mechanical shaker
- Funnel
- Filter paper with medium porosity
- Test tube

Procedure
1. Place 10 to 20 g of air-dried soil into a 250-mL bottle or flask and add 100 to 150 mL of distilled water.
2. Place a lid or stopper on the container and place on a mechanical shaker for 15 min.
3. Filter the extract through the filter paper.
4. Place approximately 5 mL of the extract in a test tube and add an approximately equal volume of acetone. Mix the solutions. The formation of a white precipitate indicates the presence of gypsum in the soil.

Removal of Soluble Salts and Gypsum
Soluble salts and gypsum are typically removed from soils and sediments by one or more rinses with distilled water.

Equipment and Materials
- 250-mL plastic centrifuge bottles with lids
- Ultrasonic probe or vortex mixer effective at mixing and dispersing the sample
- Centrifuge with head for 250-mL bottles

Note: The centrifuge used in all of the procedures outlined in this chapter should be equipped with swing out buckets (i.e., the walls of the containers swing to a position perpendicular to the rotation axis when spinning). Additional details concerning centrifuge equipment are discussed in Section VII.C of this chapter.

Procedure
1. After rinsing the soil with distilled water at least once, transfer enough soil to provide a thickness of approximately 2 to 3 cm to a 250-mL centrifuge bottle labeled with the sample identification number.
2. Add 100 mL of distilled water to the centrifuge bottle and mix. Effective dispersion and mixing of the sample may be accomplished quickly using an ultrasonic probe. If an ultrasonic probe is not available, it is critical to ensure that the sample has been effectively mixed. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Centrifugation speeds will be given in revolutions per minute (rpm) throughout this chapter, rather than as relative centrifugal force or units of gravity (g). Most centrifuges only have settings in rpm. If the centrifuge you are using only measures a g force, then refer to the owner’s manual or a variety of nomographs available to obtain the conversion factor based on the radius of the centrifuge rotor. It is important to make sure that the centrifuge is balanced. Decant the clear supernatant and check for the presence of gypsum or salts by treating an aliquot with acetone or measuring the EC. If the EC is 1 dS m⁻¹ or less, the soluble salts and gypsum have been removed from the sample.
3. Repeat treatment with water until the salts and gypsum are dissolved. If the soil is dispersed, centrifuge again or add a few drops of saturated NaCl or 0.5 M MgCl₂ solution to induce flocculation (Kunze and Dixon, 1986).
Caution: Never centrifuge bottles with rubber stoppers in place or the stopper may end up inside the bottle. Always use a screw on lid; the top of the centrifuge bottle may be wrapped with Teflon tape to prevent leakage during mixing.

Test for the Presence of Carbonates

The most common carbonate minerals occurring in soils include calcite (CaCO$_3$), dolomite [CaMg(CO$_3$)$_2$], and magnesium-calcite [Ca$_2$Mg$_{1-x}$CO$_3$] (Doner and Grossl, 2002). The presence of carbonates may be determined by the addition of dilute hydrochloric acid (~1 M HCl) to the soil (Schoeneberger et al., 2002). The HCl should be added using a medicine dropper; only one or two drops are necessary for the reaction of carbonates with the acid to occur. If effervescence or bubbling occurs with the addition of HCl, this indicates that CaCO$_3$ or other carbonate minerals are present. Calcite reacts faster than dolomite when acid is added to the soil (Doner and Grossl, 2002). In fact, dolomite may show no effervescence with acid unless it is powdered.

Removal of Carbonates

Carbonates are commonly removed from soils and sediments by treatment with Na-acetate adjusted to a pH of 5. Soluble salts may also be removed as part of this treatment (Kunze and Dixon, 1986). Other methods of removing carbonates are discussed in the chapter on selective dissolution techniques for mineral analysis of soils and sediments (Shang and Zelazny, 2008, this volume).

Reagents

- Sodium acetate (CH$_3$COONa·3H$_2$O), 1 M adjusted to pH 5 with glacial acetic acid
- Sodium hydroxide (NaOH), 1 M

Equipment

- 250-mL plastic centrifuge bottles with lids
- Stirring rods
- 100-mL graduated cylinder
- Water bath set at 80°C
- Centrifuge with head for 250-mL bottles

Procedure

1. Weigh out a sufficient amount of sample to provide the desired amount (5–10 g) of clay (<2 μm), and transfer it to a 250-mL centrifuge bottle labeled with the sample identification number. The sample should make a layer in the bottom of the centrifuge bottle that is no more than 1.5 cm thick.

2. Add 50 mL of the pH 5 acetate solution to the centrifuge bottle, mix, and place in the water bath. Check for effervescence to confirm destruction of carbonates. After the bubbling stops, place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. It is important to make sure that the centrifuge is balanced. Decant and discard the clear supernatant fluid.

Caution: It is important that the sample not be allowed to evaporate to dryness on the water bath during any of the pretreatment procedures utilized.

3. Repeat Step 2.

4. Add 15 mL of distilled water to the centrifuge bottle, mix, and adjust to a pH of 9 to 10 with 1 M NaOH. The pH may be checked using pH indicator paper. Place the
bottle in the centrifuge and centrifugate at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid. If the suspension is not flocculated, add a few drops of saturated NaCl to the centrifuge bottle, mix, and centrifugate at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.

Techniques for Analysis of Soluble Salts, Gypsum, and Carbonates

When studying soils with accumulations of soluble salts, gypsum, and/or carbonates, it is possible to identify and quantify these constituents using XRD and other analyses. When these mineral constituents are visible in the soil profile, they can be physically extracted using dental tools and analyzed separately (Buck and Van Hoesen, 2002; Buck et al., 2006b). Once collected, the samples should be transported and stored under conditions similar to those existing at the field site because many of these minerals can hydrate, dehydrate, dissolve, and/or precipitate during storage. Therefore, it is also recommended that the samples be analyzed as quickly as possible after sampling.

Before XRD analyses, the physically separated salt minerals are crushed dry using a mortar and pestle and prepared as a powder mount. Scanning electron microscopy–energy dispersive spectrometer (SEM–EDS) analyses are recommended in conjunction with XRD analyses. Important salt mineral phases within the soil may not be detected by XRD analysis alone because many of these minerals have peaks similar to or overlapping common soil minerals such as feldspar and quartz. Additionally, the identification of accessory minerals (those that constitute <5% of the soil) is difficult because the high-intensity peaks of accessory minerals are often overprinted by the numerous low-intensity peaks of the dominant minerals.

For SEM–EDS analyses, the physically separated salt minerals are mounted using carbon tape or carbon glue and coated with either gold or carbon. Individual Stage I snowballs (0.5–3.0 mm powdery spheres of pedogenic gypsum) can be placed directly on aluminum or brass mounts as small peds to facilitate accurate assessment of the physical relationship of different minerals with the soil. If small peds are mounted, sketching a map showing the locations of the salt minerals before coating with gold or carbon is recommended. This will assist in finding the desired locations during the SEM–EDS analyses. Thin sections of soils containing salt minerals can also be used for petrographic and/or SEM–EDS analyses. However, all thin-section preparation techniques need to be carefully performed without using heat or water to prevent the loss or alteration of the minerals in question. Therefore epoxy materials must not contain water, nor should heat be used to cure the epoxy. Direct cutting of the soil samples (when indurated) and/or of the epoxied samples (when not indurated) should only be performed dry or with oil-based saws. After a sample has been prepared for SEM–EDS analyses, it must be coated with a conductive material. Coating with gold should be used if carbonate minerals are believed to be present because of overlapping peaks during EDS analyses; carbon coating should be used if analyses of phosphorus-containing minerals is desired. Separate subsamples must be coated, one with gold and one with carbon, if analyses of both carbonate and phosphate minerals are needed. The amount of gold or carbon coating utilized for salt mineral analyses is important and also varies between samples. Charging, the accumulation of negative charge on a nonconductive sample or sample that is not properly grounded, during SEM analyses is a common problem when studying salt minerals. Charging may interfere with image formation and analysis because of beam deflection, but may be minimized by careful mounting and coating to ensure effective contact for the electrons across the sample surface. This often requires additional gold or carbon coating or additional carbon glue be added to the
base or sides of the sample. However, excess coating can result in high intensity gold or carbon peaks, rendering the EDS analyses useless. Therefore, careful sample preparation is critical to obtain quality SEM images and EDS analyses.

In addition to timely analysis of the collected samples, we recommend that all the necessary SEM–EDS data be collected during the first analysis. As stated above, these minerals may hydrate, dehydrate, dissolve, or precipitate during storage. Furthermore, the gold or carbon coating on the mounted samples often fractures, and samples may become detached from their mounts, making additional analyses impossible.

The XRD and SEM–EDS analyses described above require that salt minerals are present in visible accumulations within the soil, most commonly as surficial salt crusts, Stage I snowballs, Stage II nodules, or as Stage III indurated horizons (Buck and Van Hoesen, 2002; Buck et al., 2006b). These different morphologies of pedogenic gypsum are very similar to morphologies formed by pedogenic calcium carbonate and described by Gile et al. (1966) as Stage I, II, and III (Reheis, 1987; Buck and Van Hoesen, 2002). Additionally, in some instances, salt minerals may only occur as individual crystals within the soil matrix making identification by XRD much more difficult. In such cases, SEM–EDS analyses should enable determination of mineral habit and elemental weight percentage so that a reasonable interpretation of the mineralogy can be made. However, these analyses may be time-consuming because of the difficulty inherent in trying to visually locate salt minerals in the midst of the more common minerals during SEM analyses. Backscattered electron microscopic analysis is particularly useful if the minerals contain high atomic number elements such as barium. Water soluble elemental analyses may also provide additional data for interpretation of possible mineral phases.

**REMOVAL OF ORGANIC MATTER**

Once the soluble salts, gypsum, and carbonates have been removed from a sample, if necessary, the next step is to remove the organic matter. If soil and sediment samples contain organic matter, they are typically treated to remove it before clay mineralogical analyses. The presence of organic matter may result in aggregation of soil particles, and its removal is necessary if subsequent analyses require sample dispersion (Kunze and Dixon, 1986). Organic matter may also cause an exothermic reaction between 250 and 500°C in DTA studies. Additionally, organic matter may cause background interference and prevent parallel orientation of clay minerals during preparation of slides for XRD analysis (Douglas and Fiessinger, 1971).

Removal of organic matter from soils is generally accomplished using either hydrogen peroxide (H$_2$O$_2$) or sodium hypochlorite. These two methods are described in the following sections. See Mikutta et al. (2005) for a comprehensive review and history of these methods.

**Hydrogen Peroxide (H$_2$O$_2$) Method**

The effective use of H$_2$O$_2$ for removal of organic matter requires acidic conditions. Therefore, soluble salts and carbonates that add alkalinity to the soil are removed before removal of organic matter. Artificial formation of calcium oxalate is common when carbonates are present during H$_2$O$_2$ oxidation of organic matter (Farmer and Mitchell, 1963; Mikutta et al., 2005). A small amount of glacial acetic acid may be added to control the oxidizing effects of manganese oxides, if present, in the sample (Kunze and Dixon, 1986).
Manganese oxides are very strong oxidizing agents with oxidizing potentials higher than \( \text{O}_2 \) (Dixon and White, 2002).

**Reagents**
- Hydrogen peroxide (\( \text{H}_2\text{O}_2 \)), 30%
- Acetic acid or acetone, reagent grade
- Sodium chloride (\( \text{NaCl} \)), 1 M
- Sodium chloride (\( \text{NaCl} \)) saturated solution: Shake 80 g of reagent-grade \( \text{NaCl} \) in 200 mL of water.

**Equipment**
- 250-mL plastic centrifuge bottles
- Stirring rods
- Ultrasonic probe or vortex mixer
- 5-mL pipette (graduated)
- Water bath set at 80°C
- Centrifuge with head for 250-mL bottles

**Procedure**

1. Add approximately 20 mL of water to the soil sample remaining in the centrifuge bottle after soluble salts, gypsum, and carbonates have been removed. Heat the bottle to 80°C in a water bath or on a steam table.
2. Add approximately 1 mL of \( \text{H}_2\text{O}_2 \) to the centrifuge bottle. When the oxidation of organic matter begins, as indicated by frothing, add a few drops of acetic acid or acetone and stir constantly.
   **Caution:** Severe chemical burns can result if \( \text{H}_2\text{O}_2 \) comes in contact with the skin. Affected areas should be rinsed thoroughly with water immediately after contact.
3. When the frothing has subsided, additional \( \text{H}_2\text{O}_2 \) should be added to the centrifuge bottle in approximately 5-mL increments. The solution should be stirred constantly, and drops of acetic acid or acetone added to control frothing.
4. Repeat Step 3 until a total of 25 mL of \( \text{H}_2\text{O}_2 \) has been added.
   **Note:** The rate at which the \( \text{H}_2\text{O}_2 \) is added depends on the violence of the frothing that occurs. Soils with high organic matter contents will froth more than those with low organic matter contents. Some soil may be lost if the solution overflows the bottle during the reaction between \( \text{H}_2\text{O}_2 \) and organic matter. If Mn oxides are present, the \( \text{H}_2\text{O}_2 \) is decomposed so that it is less effective (or ineffective) at oxidizing the organic matter. Fizzing, rather than frothing, results during the exothermic reaction of Mn oxides, and \( \text{H}_2\text{O}_2 \), and may cause the suspension to overflow.
5. The oxidation of organic matter is assumed to be complete when a total of 25 mL of the 30% \( \text{H}_2\text{O}_2 \) has been added to the centrifuge bottle, and the frothing reaction subsides to moderate bubbling. The bubbling is the result of the thermal decomposition of \( \text{H}_2\text{O}_2 \). This will usually take several hours.
6. After \( \text{H}_2\text{O}_2 \) is decomposed and bubbling has subsided, place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. It is important to make sure that the centrifuge is balanced. Decant and discard the clear supernatant fluid. If the sample disperses, add 1 or 2 mL of saturated \( \text{NaCl} \) solution and centrifuge again.
7. Add 50 mL of 1 M \( \text{NaCl} \) solution to the centrifuge bottle, disperse the soil, and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.
8. Repeat Step 7.
9. The sample is now ready for removal of oxides or dispersion and particle-size fractionation.

**Sodium Hypochlorite Method**

Sodium hypochlorite may also be used to remove organic matter from soils and sediments before clay mineralogical analyses (Anderson, 1963). The use of sodium hypochlorite is generally preferred because degradation of clay minerals by \( \text{H}_2\text{O}_2 \) treatments has previously been documented (Douglas and Fiessinger, 1971).

**Reagents**
- Sodium hypochlorite (unbuffered household bleach), adjusted to pH 9.5 or less with 0.2 M hydrochloric acid (HCl) shortly before use. Determine whether the bleach is buffered or not by attempting to adjust the pH. If it is buffered, a large volume of HCl will need to be added to change the pH, and the pH will drift back to the original value (~11). Several brands of bleach may need to be tested to find one that is unbuffered.
- Hydrochloric acid (HCl) 0.2 M
- Sodium hydroxide (NaOH) 1 M
- Sodium chloride (NaCl) 0.1 M
- 95% Ethanol

**Equipment**
- 250-mL plastic centrifuge bottles
- Stirring rods
- 100-mL graduated cylinder
- Ultrasonic probe or vortex mixer
- Water bath set at 80°C
- Centrifuge with head for 250-mL bottles

**Procedure**
1. Add 50 mL of sodium hypochlorite (bleach) adjusted to pH 9.5 or less to the soil in the 250-mL centrifuge bottle, mix, and disperse the sample. Place the bottle in an 80°C water bath for 15 min.
2. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. It is important to make sure that the centrifuge is balanced. Decant and discard the clear supernatant fluid.
3. Repeat Step 2 twice for a total of three treatments with bleach, or until organic matter has been completely removed as indicated by a clear, uncolored solution. **Note:** A pink color may result if the soil contains manganese oxides. The pink coloration should be disregarded in terms of organic matter removal.
4. Add 50 mL of 0.1 M NaCl to the centrifuge bottle, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant solution.
5. Repeat Step 4 one more time. The rinse with 0.1 M NaCl serves to wash out the products of organic matter decomposition. If the sample disperses, add a few drops of saturated NaCl solution.
6. Add 25 mL of distilled water to the centrifuge bottle, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at 2000 rpm for 10 min. Check the supernatant for suspended clay. If the supernatant contains suspended clay, the sample is now ready for removal of oxides or dispersion and particle-size fractionation.
7. If the supernatant was clear, decant it and add 25 mL of ethanol. Mix and disperse the sample. Place the bottle in the centrifuge and centrifuge at 2000 rpm for 10 min. Check the supernatant for suspended clay. If the supernatant contains suspended clay, the sample is now ready for removal of oxides or dispersion and particle-size fractionation. If the solution is clear, decant and discard it and then proceed with additional pretreatments and/or dispersion and fractionation.

**Note:** During any of the pretreatment procedures described in this chapter, a high-speed centrifuge (>10,000 rpm) may be used to bring down dispersed clays when the other flocculating methods are not working.

**REMOVAL OF IRON OXIDES**

The presence of free oxides and hydrous oxides of Al, Fe, Mn, Si, and Ti may make it difficult or impossible to disperse soil samples before fractionation procedures because these compounds may act as cementing agents. These compounds may exist in crystalline, poorly crystalline (short-range order), and/or noncrystalline (amorphous) forms in soils as a result of pedogenic processes. In particular, the removal of free iron oxides enhances the parallel orientation of layer silicate clays and allows for the detection of X-ray diffraction peaks that might otherwise be obscured (Mehra and Jackson, 1960).

Most of our existing information on Al, Fe, Mn, Si, and Ti oxides and hydroxides in soils has been derived from selective chemical dissolution (SCD) analyses. Selective chemical dissolution analyses may be performed either on the untreated <2-mm soil fraction or the clay (<2-µm) fraction. Detailed information concerning SCD techniques and their applicability to Al, Fe, Mn, Si, and Ti oxides and hydroxides are provided by Shang and Zelazny (2008, this volume).

**Reagents**

- Citrate buffer solution, comprised of the following two reagents:
  - Sodium citrate, 0.3 M Na$_2$C$_6$H$_5$O$_7$·2H$_2$O (88 g L$^{-1}$)
  - Sodium bicarbonate, 1 M NaHCO$_3$ (84 g L$^{-1}$)
  - Premix these reagents at a ratio of 8:1 citrate/bicarbonate solutions (i.e., 40 mL of 0.3 M sodium citrate to 5 mL of 1 M sodium bicarbonate).
- Sodium dithionite powder (Na$_2$S$_2$O$_4$)
- Sodium chloride (NaCl) 1 M
- Saturated NaCl solution
- Acetone, reagent grade

**Equipment**

- 250-mL plastic centrifuge bottles
- Stirring rods
- 100-mL graduated cylinder
- Ultrasonic probe or vortex mixer
- Water bath set at 80°C
- Centrifuge with head for 250-mL bottles
- Analytical balance

**Procedure**

**Note:** This procedure produces a strong “rotten egg” smell with irritating fumes and should be conducted under a ventilated fume hood.

1. Centrifuge and discard the clear supernatant from the previous treatment for removal of organic matter or soluble salts.
2. Add 10 to 15 mL of saturated NaCl solution and 150 mL of distilled water to the centrifuge bottle, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. It is important to make sure that the centrifuge is balanced. Decant and discard the clear supernatant fluid.
3. Add 100 ±5 mL of citrate buffer solution and heat the bottle to 75 to 80°C in a water bath.
4. When the sample reaches 75 to 80°C, add approximately 1 g of Na$_2$S$_2$O$_4$ to the bottle. Stir continuously for 1 min, and then occasionally for 5 min.
5. Add a second 1-g portion of Na$_2$S$_2$O$_4$ to the bottle and stir as described above.
6. Add a 2-g portion of Na$_2$S$_2$O$_4$ and stir as described above.
7. After 10 to 15 min, remove the bottle from the water bath and cool. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.
8. Add 10 mL of saturated NaCl solution, 10 mL of acetone, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.
9. Add 100 mL of 1 M NaCl solution to the bottle, mix, and disperse the sample. Place the bottle in the centrifuge and centrifuge at about 1500 rpm for 5 min. Decant and discard the clear supernatant fluid.
10. Repeat Step 9.

**REMOVAL OF SHORT-RANGE ORDER ALUMINOSILICATE MATERIALS**

The identification of short-range-order aluminosilicate materials, such as allophone and imogolite, in a variety of soils has resulted in the development of several different techniques that attempt to quantify the abundance and physical and chemical characteristics of these materials. Characterization and quantification of these materials is important because of their large specific surface areas and high proportion of reactive sites, which influence the surface chemistry of soils (Dahlgren, 1994).

Most of our existing information on short-range order aluminosilicates in soils has been derived from SCD analyses. For example, allophone and imogolite are differentiated from other poorly ordered inorganic phases and from organically bound Al based on their relative resistance to dissolution by various chemical reagents (Dahlgren, 1994). Selective chemical dissolution analyses may be performed either on the untreated <2-mm soil fraction or the clay (<2-μm) fraction. For additional information concerning SCD techniques and their applicability to short-range order materials, please refer to Shang and Zelazny (2008, this volume) and Dahlgren (1994).

**PARTICLE-SIZE FRACTIONATION**

Particle-size fractionation is an integral component of soil characterization. It is one of a number of concentration techniques applicable to soil mineralogical analysis and is by far the most routinely used (Laird and Dowdy, 1994). Fractionation of particle sizes increases detection sensitivity because specific minerals tend to be more prevalent in specific size ranges. Furthermore, mineralogical assessment of the clay fraction is often the sole objective of the analyst. Other concentration techniques, including density or magnetic fractionation, are also typically performed on specific particle-size fractions.

The increments of particle size in the fractionation procedure are discretionary. A common practice is to fractionate the <2-mm soil material into sand (2–0.05 mm), silt...
preparing soils (0.05–0.002 mm [50–2 µm]), and clay (<0.002-mm [<2-µm]). These particle-size ranges are specified by the USDA, but other standard size ranges may also be used. Further fractionation may be necessary for some specialized mineralogical assessments. A method will be presented below for particle-size fractionation using USDA specifications for sand, silt, and clay, as well as methods for further fractionation of these three major divisions.

Principles

All particle-size fractionation procedures are designed to prevent, or more realistically, minimize particle aggregation so that discrete particles behave independently. Soils tend to have aggregated particles due to the presence of organic matter, metal oxides, carbonates, and salts—these components can bind primary particles together. The procedures described at the beginning of this chapter are designed to remove these aggregating agents before particle-size fractionation and facilitate dispersion of the sample.

The first step in the particle-size fractionation procedure is generally to separate the clay (<2 µm) from the silt (0.05–0.002 mm [50–2 µm]) and the sand (2–0.05 mm [2000–50 µm]). Fractionation is accomplished by differential settling of the particles in a liquid. Centrifugation is routinely used to increase the settling rates. Sedimentation in large beakers is possible, but is much slower for separating clay from silt. Several variables, including particle size, affect the rate of particle settling in viscous media and must be accounted for or controlled when performing any fractionation procedure.

The relationship between the settling velocity of a particle within a liquid and the variables affecting it is expressed by Stokes’ Law (Stokes, 1851),

\[ V = g(s_p - s_l)D^2/1.8\eta \]  

where \( V \) is terminal particle velocity (cm s\(^{-1}\)) in a liquid medium of density \( s_l \), \( g \) is acceleration due to gravity (980 cm s\(^{-2}\)), \( s_p \) is particle density (g cm\(^{-3}\)), \( D \) is the “equivalent spherical diameter” of the particle (cm), and \( \eta \) is the viscosity of the liquid (Pascal seconds [Pa-s]). This equation shows that settling velocity is directly proportional to force applied, difference between particle density and liquid density, and particle diameter; and inversely proportional to liquid viscosity.

The time in seconds \( (t_{sec}) \) for a particle of diameter \( D \) (cm) to settle a distance \( h \) (cm) is given by the following equation (Jackson, 1979):

\[ t_{sec} = 1.8\eta h/[g(s_p - s_l)D^2] \]  

This relation can be used to calculate settling times for specific particle sizes and settling distances of interest to the analyst, using a computer spreadsheet (Table 2–1).

Settling times under centrifugal acceleration must be calculated using an integrated form of Stokes’ Law (Svedberg and Nichols, 1923; modified by Jackson, 1979), since acceleration increases as a particle settles and the rotation axis increases,

\[ t_{min} = 6.3 \times 10^8\eta \log_{10}(R/S)/[(rpm)^2(D_m)^2(s_p - s_l)] \]  

where \( t_{min} \) is settling time (min), \( R \) is the distance (cm) from the rotation axis (i.e., the center point of the centrifuge head) to the top of the sediment in the tube when the walls of the containers are perpendicular to the rotation axis, \( S \) is the distance (cm) from the rotation axis (i.e., the center point of the centrifuge head) to the top of the suspension, rpm is revolutions per minute, and \( D_m \) is particle diameter (µm). The \( R \) and \( S \) distances may be different for different centrifuges and should be measured prior to calculating settling times. This equation can also be set up in a spreadsheet to generate a table of times and corresponding rpm values (Table 2–2).
Some variables affecting particle settling rate can be controlled for the purpose of fractionation, while others cannot. The variable $g$ can be maintained as constant or accounted for mathematically, as can the variables $s_i$ and $n$ if temperature is controlled. However, the particle density can only be set at some reasonably representative value, which is commonly taken to be the density of quartz (2.65 g cm$^{-3}$). Obviously, minerals with different densities occur in soils, and fixing the density at a single value precludes precisely defined particle-size fractions.
Another unavoidable simplification of the Stokes model is the assumption that particles are spheres. Many soil mineral particles in the fine size fractions are platy rather than spherical, and settle at a slower rate than a sphere of equivalent volume. There is a “hidden” temperature effect on settling time, since \( \eta \) and (to a lesser extent) \((s_p - s)\) vary with temperature. However, concern about small temperature variations (e.g., \(<3^\circ C\)) in light of the uncertainties of particle densities and shapes is probably unwarranted. There is also a slight error resulting from the time needed to bring the centrifuge up to speed and for it to slow down at the end. Particle-size fractions separated using the principles of Stokes’ Law are therefore unavoidably nominal and operationally defined. Nonetheless, particle-size fractionation is a vitally useful procedure in soil mineralogical analysis, assumptions and simplifications notwithstanding.

### Pretreatments and Dispersion

Pretreatments to selectively remove aggregating agents such as soluble salts, carbonates, oxides, and organic matter were described above. Tradeoffs in using pretreatments are (i) the removal of components that are themselves mineral constituents of the soil (Jones and Malik, 1994; Buck et al., 2006b) and (ii) the risk of altering other minerals (e.g., Harward and Theisen, 1962; Harward et al., 1962; Douglas and Fiessinger, 1971; Brewster, 1980). The decision to use a given pretreatment depends on whether or not the minerals of primary interest for a particular soil can be adequately assessed without the selective removal of an aggregating component. Ultrasonic techniques (Edwards and Bremner, 1967; Busacca et al., 1984) have been used as an alternative to chemical pretreatments in promoting dispersion.

Particle-size fractionation requires that particles be dispersed and free to settle independently in a liquid medium. “Particles” ideally are individual minerals, but they can also be strongly cemented aggregates. For example, rock fragments (e.g., shale) and oxide-cemented nodules can occur in the sand and silt fractions. Thus, fractionation of particles can be operationally dependent on the severity of the dispersion approach.

Electrostatic attraction among fine particles is another phenomenon that can impede effective dispersion. The degree of attraction is inversely related to the magnitude of charge for mineral surfaces, as well as to the valence and hydrated radius of counterions. Dispersion of soils having a significant amount of mineral surfaces with pH-dependent charge can be promoted by adjusting the pH away from the “point of zero net charge” of the prevalent pH-dependent components. Such an adjustment increases surface charge, and hence electrostatic repulsion and dispersion. Dispersion of negatively charged minerals can also be promoted, for both permanent- and variable-charge minerals, by saturating with monovalent cations with large hydrated radii (e.g., Na+). Low ionic strength is a requirement for effective dispersion, since thickness of the diffuse double layer (counterion “cloud” that electrostatically maintains distance between particles) diminishes with increasing ionic strength.

### Methodology for Particle-Size Fractionation

The methods for particle-size fractionation must be tailored to the available equipment and the particle-size ranges of interest. The analyst must also make decisions regarding pretreatment from what is known about the sample at the outset. There is no “one size fits all” methodology for particle-size fractionation. The method described in this section involves fractionation within three nominal particle-size ranges (2–0.05 mm, 50–2 \( \mu \)m, and \(<2 \mu \)m), but analysts can customize ranges for their objectives using a computer...
spreadsheet and the equations given above (e.g., Tables 2–1 and 2–2). Modern programmable centrifuges may also facilitate the targeting of different size ranges. This method requires use of a centrifuge for which the walls of the containers swing to a position perpendicular to the rotation axis. The tube used would be filled to the 10-cm mark, allowing 1 cm for sediment thickness in the bottom of the tube. Centrifugation speeds and times shown in Tables 2–1 and 2–2 are based on an \( R \) value (see Eq. [3]) of 25 cm and an \( S \) value of 16 cm, but other dimensions of \( R \) and \( S \) can be specified, depending on the centrifuge model. Angle-head centrifuges require an adjustment to the \( S \) measurement because of the (i) shorter distance from the top of the suspension to the top of sediment and (ii) the turbulent and convective flow that occurs when the walls of the container are oblique to the rotation axis (Jackson, 1979). Angle-head centrifuges require careful removal of the centrifuge tube from the centrifuge. The sloping surface of the sediment may result in instability and slumping, and hence some resuspension of the sediments.

Readers that have a centrifuge with different dimensions from those specified for the procedure presented in this chapter can modify the times and/or rpm values according to their equipment specifications or to other particle-size ranges of interest. The time that is calculated for a given rpm value and particle diameter is the time it takes (ideally) for all particles of that diameter to settle, such that only particles less than that diameter would still be in suspension. For example, 2-\( \mu \)m diameter spherical particles of 2.65 g cm\(^{-3} \) would settle 9 cm (allowing for 1 cm of sediment from a 10-cm suspension) in 1.7 min at 1000 rpm and the other conditions specified in Table 2–2, after which the suspension could be siphoned off and labeled as “<2 \( \mu \)m.” Greater initial rpm values times are specified in this chapter to remove the bulk of the clay particles, most of which are much smaller than 2 \( \mu \)m. Repeated spins at 1000 rpm are performed to remove clay close to the 2-\( \mu \)m limit. The repetition is necessary because for any given spin, most particles (those not at the top of the suspension) have a travel path of less than 9 cm to the bottom of the container.

**Reagents**

- “pH-10 water,” sodium carbonate (\( \text{Na}_2\text{CO}_3 \)) solution, pH 9.5 (\( \sim 0.1 \) g \( \text{Na}_2\text{CO}_3 \) L\(^{-1} \))
- Sodium chloride (\( \text{NaCl} \)), 1 M

**Note:** If your samples are in the neutral to alkaline pH range, distilled or deionized water may be used in place of the pH-10 water for sample dispersion. The pH-10 water is used to facilitate dispersion of acidic soil samples. Also, ultrasonic techniques (Edwards and Bremner, 1967; Busacca et al., 1984) can be used for dispersion if there is concern about mineral destabilization at elevated pH.

**Equipment and Materials**

- Centrifuge
- pH meter
- Balance
- 53-\( \mu \)m sieve (300 mesh)
- Large funnel
- Ring stand
- Centrifuge containers (60-mL tubes or 250-mL bottles) with caps
- 1000- and 2000-mL beakers
- Sample fraction storage containers
Dispersion Procedure

Sodium Saturation

This step may be eliminated if Na-based pretreatments such as those described in previous sections of this chapter are used:

1. Place a layer of soil (<2 mm) approximately 1.5 cm thick in the centrifuge container.
2. Add 1 M NaCl solution to a 9-cm height (or height appropriate for the equipment being used) in the containers. Mark the containers for this height.
3. Centrifuge for 5 min at 2000 rpm.
4. Decant and discard the clear supernatant; then repeat Steps 3 and 4 twice more (i.e., a total of three washes with NaCl).
5. Decant the last NaCl wash supernatant.
6. Add deionized water to the 9-cm mark, and centrifuge as before (2000 rpm for 5 min).
7. Repeat the deionized water rinse until the supernatant is no longer clear (appears turbid) after centrifugation. Save this turbid suspension by siphoning it into a 2000-mL beaker labeled as “<2 µm” along with sample identification. You will add to this clay suspension as you perform particle-size fractionation. Save the Na-saturated sediment for the next fractionation step.

Separation of Clay (<2 µm)

The following procedure is based on the use of a 250-mL centrifuge bottle, no more than 1.5 cm of sediment, and 9 cm to the top of the suspension in the bottle. As stated above, the analyst needs to check the equipment being utilized for fractionation purposes and adjust the equation for calculating centrifuging times as appropriate.

1. Make a mark on the centrifuge bottle at 9 and 3 cm from the bottom of the bottle. The sediment in the bottle should be approximately 1.5 cm in thickness.
2. Add deionized water up to the 9-cm mark on the bottle.
3. Mix thoroughly and then centrifuge at 500 rpm for 6 min.
4. Siphon the <2-µm fraction in suspension from 3 cm above the bottom of the bottle into a 1000- or 2000-mL beaker. If the sample is dispersed, the suspension should be cloudy or turbid. If the sample is not dispersed, as indicated by a clear suspension, the clear supernatant can be poured off and discarded. If the sample fails to disperse, add one drop of saturated NaCl solution, remix the sample, and centrifuge. Care should be taken to ensure that no portion of the sediment in the bottom of the bottle, or below the 3-cm mark, is siphoned off or discarded during this procedure.
5. Repeat Steps 2 through 4 several times until the suspension is clear or only slightly cloudy following centrifugation. In our experience, eight repetitions are generally adequate to effectively separate the <2-µm fraction. Excessive repetitions may produce clay mechanically.
6. The suspension in the beaker now contains the Na-saturated clay (< 2-µm) fraction from your sample.

Flocculation of the Clay

The clay fraction is suspended in a rather large volume of water following fractionation. The water content should be reduced for storage and subsequent analyses. Flocculation
(chemically induced aggregation) of clay in suspension greatly increases the rate of particle settling, and produces a clear supernatant that can be siphoned off to achieve the volume reduction. There are several procedures to flocculate clay in suspension. The best procedure depends somewhat on the characteristics of the sample. The easiest method is to add saturated NaCl to the suspension, stir, cover, and allow to settle for several hours. The increase in ionic strength with the addition of the saturated NaCl reduces double layer thickness, allowing particles to approach closely enough so that forces of attraction can induce flocculation. However, some samples do not readily flocculate with NaCl. Another method is to adjust the pH close to the zero point of net charge for the major mineral components anticipated in the sample. For example, adjusting the pH to about 4.0 is sometimes effective in inducing flocculation for clay from acidic soils dominated by kaolinite. The pH should be adjusted back to a range suitable for stable storage once flocculation has been achieved to prevent mineralogical and other sample changes caused by the acid used for flocculation.

**Separation of the Sand (2000–50 µm) and Silt (50–2 µm)**

The silt fraction may be separated from the sand using centrifugation, gravitational settling, or wet sieving. The settling depths and times may be calculated using nomographs and equations provided in Jackson (1979) or Tanner and Jackson (1947), or by developing a spreadsheet to produce a table similar to Table 2–1 using the appropriate diameters and other variables.

**Centrifugation**

1. If using centrifugation, take the bottle containing the sand and silt and add pH-10 water to the 9-cm mark, shake, and centrifuge at the calculated rpm and time. The pH-10 water is used to suspend the silt in solution.
2. Following centrifugation, siphon the suspended silt off into a 1000- or 2000-mL beaker.
3. Repeat Steps 1 and 2 until the supernatant liquid is nearly clear. In our experience, three to eight repetitions are required to adequately separate the silt from the sand. Use deionized water instead of the pH-10 water for the last two to three centrifugations to avoid leaving a Na₂CO₃ residue in the sand and the silt.
4. Allow the silt in the beaker to settle until the supernatant is clear. The clear supernatant can be siphoned off and discarded, leaving the wet silt, which should be stored wet in an appropriate container.
5. Following removal of the silt, the sand and excess water remaining in the centrifuge bottle can be transferred to a beaker. The excess water can be evaporated on a steam bath. If abundant mica is present, the moist sand should be stirred after most of the free water has evaporated. This stirring helps prevent a mat of mica from forming. The sand should then be oven-dried at 105°C for about 2 h. After drying, the sand can be stored dry in a glass vial or other storage container.

**Gravitational Settling**

1. If using gravitational settling, transfer the sand and silt to a 1000-mL beaker. After the sand and silt are in the beaker, add pH-10 water to suspend the silt in solution. Mix and allow the suspension to stand for the calculated time for the depth used.
2. Siphon off the suspended silt and collect it another beaker.
3. Repeat Steps 1 and 2 until the supernatant is nearly clear. Use deionized water instead of the pH-10 water for the last two to three settleings to avoid leaving a Na₂CO₃ residue in the sand and the silt.
4. Allow the silt in the beaker to settle until the supernatant is clear. The clear supernatant can be siphoned off and discarded, leaving the wet silt, which should be stored wet in an appropriate container.

5. Following removal of the silt, the sand and excess water remaining in the beaker can be evaporated on a steam bath. If abundant mica is present, the moist sand should be stirred after most of the free water has evaporated. This mixing helps prevent a mat of mica from forming. The sand should then be oven-dried at 105°C for about 2 h. After drying, the sand can be stored dry in a glass vial or other storage container.

**Wet Sieving**

It is also possible to separate the sand from the silt by wet sieving following the removal of the clay. A 53-, 56-, 44-µm (270-, 300-, or 325-mesh) sieve is commonly used to collect the sand.

1. Set up a funnel on a ring stand and place a 1000-mL beaker beneath the funnel. Place a 53-, 56-, or 44-µm sieve in the funnel.

2. Add pH-10 water to the centrifuge bottle containing the sand and silt, cap the bottle and mix thoroughly. Pour the suspension onto the sieve. Transfer the remaining material in the centrifuge bottle, including any material adhering to the lid, to the sieve using jets of pH-10 water from a wash bottle. If you are working with a very sandy soil, you may want to add the sand gradually so that the sieve does not become clogged.

3. After all the material has been transferred to the sieve, continue to wash the dispersed soil through the sieve using jets of pH-10 water from a wash bottle. When the effluent begins to clear, switch to deionized water and continue washing until the effluent is clear. A pliable rubber policeman may be used to gently crush any aggregates, using caution not to damage the sieve.

4. Remove the beaker containing the silt from under the sieve. The silt can be concentrated and stored as described above. Quantitatively transfer the sand on the sieve to a beaker and dry at 105°C for approximately 2 h. After drying, the sand can be stored dry in a glass vial or other storage container.

**FREEZE-DRYING**

After completing the necessary pretreatments and particle-size fractionation, freeze-drying of the clay suspensions is recommended for preservation. Freeze-drying is used routinely in the biological sciences to preserve naturally occurring organic and inorganic substances, and it is also effective for the preservation of clays (Malcolm, 1968). Freeze-drying involves drying frozen matter under high vacuum so that the solidified solvent or suspending medium sublimes, and a residue, typically solid, remains (Malcolm, 1968). Many organisms are killed during the freezing and subsequent freeze-drying process. Those that are still viable after the treatment are dormant in the freeze-dried residue, which contains no free moisture. Freeze-dried organic materials, therefore, may be stored for future use in a desiccator without danger of microbial degradation or physicochemical changes (Malcolm, 1968).

Clays are often preserved by air drying from aqueous suspension. During air drying, hard flakes or cakes are typically formed that are difficult to crush into a fine powder. The major advantages of freeze-dried clays are related to the fluffy, powderlike consistency that results from the freeze-drying process. Freeze-dried clays can be resuspended as col-
loidal clay instantaneously (Malcolm, 1968). Resuspension of air dried clays, in contrast, is a much more time consuming process and may require considerable effort. Additionally, freeze dried clays may be used for DTA, XRD, and for infrared (IR) analysis with minimal, if any, grinding.

Freeze-drying is not recommended for soils containing soluble salts, if salt mineralogy analyses are desired. Freeze-drying and eventual thawing can drastically alter salt mineralogy.

**Reagents**
- Silver nitrate (AgNO₃), 0.5 M
- Slurry of dry ice and ethanol for quick freezing clay suspensions

**Equipment and Materials**
- 1- to 2-L glass beakers
- Dialysis tubing
- Freeze-drying flasks (various sizes depending on volume) and adaptors
- Freeze-dryer

**Procedure**
1. After fractionation, transfer approximately one-half of the Na-saturated clay into dialysis tubing, securely attach a sample identification label, and place the tubing in a large beaker of distilled water.
2. Replace the distilled water in the beaker at least twice a day until the washing solution is free of chloride. You can test the completeness of washes for chloride removal by adding one drop of AgNO₃ solution to a separate beaker containing a sample of the rinse water. Check for the formation of a white (cloudy) AgCl precipitate. Washing is complete when no cloudy precipitate forms.

**Note:** Always handle the clay-filled dialysis tubing carefully to avoid breakage and loss of sample.

3. After dialysis has been completed, transfer the Na-saturated clay from the dialysis tubes to labeled freeze-drying flasks. The size of the flask will vary depending on the volume of clay to be freeze-dried.
4. Freeze the Na-clay in the freeze-drying flasks in a dry ice–ethanol slurry.
5. Attach the frozen flasks to the freeze-dryer in accordance with the manufacturer’s instructions.

**REFERENCES**


