

Comparison of Centrifugation and Filtration Techniques for the Size Fractionation of Colloidal Material in Soil Suspensions Using Sedimentation Field-Flow Fractionation

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Sedimentation field-flow fractionation (SdFFF) with UV detection is used to systematically investigate the effect of traditional membrane filtration and centrifugation procedures on the isolation of specific size fractions from soil suspensions. Both procedures were used to isolate the nominal <0.45 and $<0.2 \mu\text{m}$ fractions from a clay soil suspension. Results showed that the membrane filtration approach seriously underestimated the total mass of particulate matter present as compared to the centrifugation approach. This has serious implications for the interpretation of results for “colloidal” and “soluble” fractions from soil suspensions and other environmental matrices obtained using the standard membrane approach. The results also show that sedimentation FFF has great potential as a robust and relatively mild technology for studying size distributions in the “colloidal” range for soil suspensions and other aquatic matrices.

Introduction

Colloidal material (0.001 – $1 \mu\text{m}$) in soil leachate and drainage waters is an important vehicle for the transport of contaminants (1, 2) such as phosphorus species (3, 4), pathogens (5–7), persistent organic pollutants (8), and nitrogen species (9, 10). Therefore, accurate and sensitive methods for the separation of particulate and colloidal material from soil suspension samples are essential (11–13).

Conventional filtration methods have traditionally been used for the separation of dissolved and particulate fractions in environmental samples, using an operationally defined filter pore size of 0.2 or $0.45 \mu\text{m}$ as the “threshold” (14). The colloidal fraction, which spans a wider range than these

nominal pore sizes, has therefore been difficult to study. Haygarth et al. and Heathwaite et al. used membrane and ultrafiltration methods to separate different colloidal size ranges in river water and soil leachates, but found that colloids aggregated at the membrane surface (15, 16). Colloids also interact directly with the membrane, resulting in material being retained (17), and there can also be memory effects, contamination from the filter, and variable pressure across the membrane.

Many studies have used centrifugation and filtration methods sequentially to prepare soil samples (18–21). Del Castillo et al. (22) studied the difference between centrifuged and membrane-filtered soil suspensions to remove suspended material at a threshold of $<0.45 \mu\text{m}$ and then analyzed the resulting fractions for a range of elements. They found that colloid-associated properties differed between membrane filtration and centrifugation, with membrane filtration producing higher values, and therefore suggested that membrane filtration, being the simpler method, was the preferred technique for the removal of colloidal material. Douglas et al. (23) sequentially used three separation techniques: sieving, continuous flow centrifugation, and tangential flow filtration (TFF) to fractionate suspended material in river waters over the particulate and colloidal ranges. The above studies focused on how the elemental content of environmental samples differed using different separation techniques, but did not quantitatively investigate the colloidal size distribution.

To overcome the uncertainties encountered with membrane filtration, and also to be able to characterize the colloidal material, Buffle and Leppard suggested the use of “a promising new technique”, field-flow fractionation (FFF), for colloidal fractionation (17). This emerging separation technique can be used to obtain information on particle size or relative molecular mass (RMM) distributions in complex environmental matrices over the entire colloidal size range. There are many subtechniques of FFF of which sedimentation (Sd) and flow (Fl) are the most commonly used. FFFF separates molecules or particles using a cross-flow field, and the process is independent of density, whereas SdFFF separates on the basis of buoyant mass (i.e., size and density) using a centrifugal field. SdFFF has been used successfully to determine the size distribution of colloids in environmental samples such as soil and sediment solutions (24, 25). Results have been verified by collecting different size fractions and analyzing them using electron microscopy (25–27). Previous studies of soil, sediment, and river water samples have usually used SdFFF coupled with detectors such as ICP-MS to determine elemental composition with respect to different size fractions (24, 25, 27–32). Most of these studies pretreated the samples using gravity sedimentation (27) or centrifugation (24, 25, 28, 31, 32) to obtain a $<1 \mu\text{m}$ cutoff to avoid steric interferences (29).

The aim of this work was to use SdFFF with UV detection to systematically investigate the effect of traditional membrane filtration and centrifugation procedures on the isolation of specific size fractions from soil suspensions. Particle size thresholds of <0.2 and $<0.45 \mu\text{m}$ were selected to represent the two most common operational fractions isolated by traditional membrane filtration (17).

Experimental Section

Sample Preparation. All glassware and plastic bottles were prewashed overnight in 5% nutrient P-free detergent (Extran), rinsed with Milli-Q water three times, and then left overnight in 5% Extran and again rinsed with Milli-Q water three times.

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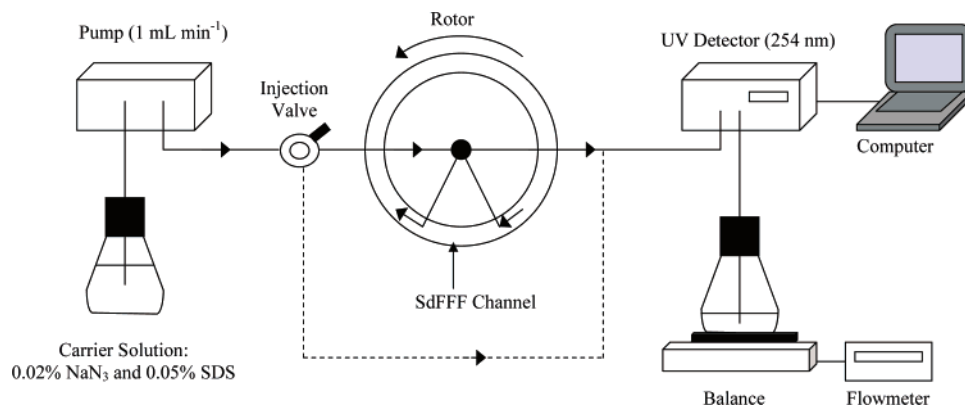


FIGURE 1. Schematic diagram of the SdFFF instrumentation. Bold lines indicate direction of carrier in run/inject mode; dotted lines indicate direction of carrier in stopflow/load mode.

A clay soil sample was previously collected from the B-horizon at Lilydale in Melbourne, Australia (28). This is a reddish brown (5YR4/3), light clay Krasnozem soil with a moderate polyhedral structure, 10–20 mm peds, rough fabric, and a firm consistence. The content was 55% clay (<2 μm), 22% silt (2–20 μm), 22% fine sand (20–200 μm), and 1% coarse sand (200 μm –2 mm) with a pH of 5.2 (in water) and 4.4 (in calcium chloride). The sample was suspended in ultrapure Milli-Q water (Millipore) and screened through a 25 μm mesh nylon sieve. The <1 μm diameter fraction was isolated by repeated centrifugation and stored at 4 $^{\circ}\text{C}$. The concentration of the <1 μm fraction was determined by drying 10 mL of sample in an oven overnight at 100 $^{\circ}\text{C}$. The weight of the dried soil sample was 5 g, giving a concentration of 50% (m/v) in the suspension. This is a highly dispersible soil sample, and from experience the particles in this concentrated suspension stay dispersed in water. This suspension was further characterized and found to contain 14 600 mg kg^{-1} aqua regia extractable iron, 19 mg kg^{-1} aqua regia extractable manganese, and 2.5 g kg^{-1} organic matter.

Fractionation of Soil Sample. The 50% (m/v) soil sample was diluted in Milli-Q water to give a 1% (m/v) suspension which was used to prepare the filtered and centrifuged <0.2 and <0.45 μm soil fractions as outlined below. Sedimentation does not occur in diluted (1% m/v) samples (28), and hence samples were diluted with Milli-Q water alone rather than adding a surfactant such as sodium dodecyl sulfate (SDS).

Filtration: Two different size fractions (<0.2 and <0.45 μm) were obtained by sequential filtration. The 1% (m/v) soil suspension (25 mL) was sequentially filtered under suction through a 0.45 μm Activon cellulose nitrate membrane filter (47 mm dia) and a 0.2 μm Whatman cellulose nitrate membrane filter (47 mm dia) using a conventional glass filtration unit.

Centrifugation: The 1% (m/v) soil suspension was pipetted into polypropylene tubes (1.7 mL volume) and placed into an Avanti 30 High-Performance benchtop centrifuge with the F2402 fixed-angle rotor. The settling time for each fraction (<0.2 and <0.45 μm) was determined using the following equations:

$$\omega = \left(\frac{2\pi}{60} \cdot \text{rpm}\right) \quad (1)$$

$$t = \frac{18\eta \ln\left(\frac{R}{S}\right)}{\omega^2 d^2 \Delta\rho} \quad (2)$$

where ω is the angular velocity of the centrifuge (rad s^{-1}), d is the particle diameter (cm), $\Delta\rho$ is the density difference between the particles and the suspension medium (g cm^{-3}), η is the viscosity of the suspension medium ($\text{g cm}^{-1} \text{s}^{-1}$)

where the viscosity of water at 20 $^{\circ}\text{C}$ is $0.010 \text{ g cm}^{-1} \text{ s}^{-1}$, t is the settling time (s), R is the distance (cm) from the axis of rotation to the level from where the supernatant is decanted from the tube, and S is the distance from the axis of rotation to the surface of the suspension in the tube (cm).

From the above equations, it was determined that the 1% m/v soil suspension (containing <1 μm particles) required a centrifugation time of 10 min at 2000 rpm (357g) at 20 $^{\circ}\text{C}$ to obtain the <0.45 μm fraction. The supernatant was decanted, and the pellet was resuspended in Milli-Q water and re-centrifuged to ensure that any remaining <0.45 μm particles retained in the pellet were recovered. This was repeated a third time, and the decanted supernatants from the three centrifuge runs were pooled. This process was repeated to obtain the <0.2 μm fraction by centrifuging the 1% m/v soil suspension at 4500 rpm (1810g) for 10 min (at 20 $^{\circ}\text{C}$).

Soil Particle Density. There is broad agreement on reported values for the density of soil mineral particles. Sainz Rozas et al. (33) assumed that the density was 2.65 g cm^{-3} , Adriano and Weber (34, 35) reported that the typical density range for agricultural soils was 2.6 – 2.75 g cm^{-3} , and arable surface soils with a high mineral content had a particle density of 2.65 g cm^{-3} , and Wienhold and Tanaka reported the same value (36). Other literature sources have assumed a particle density of 2.5 g cm^{-3} for mineral-rich sediments (29–32). A density of 2.6 g cm^{-3} (hence a density difference of 1.6 g cm^{-3}) represents a typical literature value for agricultural soils of the type used in this study and was therefore used in this work for all centrifugation and SdFFF calculations (37).

Sedimentation Field-Flow Fractionation. Details of the SdFFF instrumentation used in this work have been reported elsewhere (31). The channel dimensions were radius 15.1 cm, length 86.1 cm, breadth 2.0 cm, and width 0.0144 cm. The carrier was pumped through the channel by a ConstaMetric3000 solvent delivery system (LDC Analytical, USA) at a flow rate of 1 mL min^{-1} . The flow rate was monitored using an Ohaus Precision Plus balance and a flowmeter. A schematic diagram of the SdFFF instrumental setup is shown in Figure 1. The SdFFF carrier solution consisted of 0.05% (m/v) sodium dodecyl sulfate (SDS; VWR, Poole, England) and 0.02% (m/v) sodium azide (NaN_3 ; VWR, Poole, England) in Milli-Q water. We are confident that the low concentration of SDS used would have no effect on the particle size distribution. The carrier was degassed before use by evacuation for at least 30 min. All runs were carried out at 25 $^{\circ}\text{C}$.

A power program was used in which, after the relaxation or stopflow time, the initial field was held for time t_1 and then decayed to a holding field where the time constant t_a determined how rapidly the field decayed (38). The constants

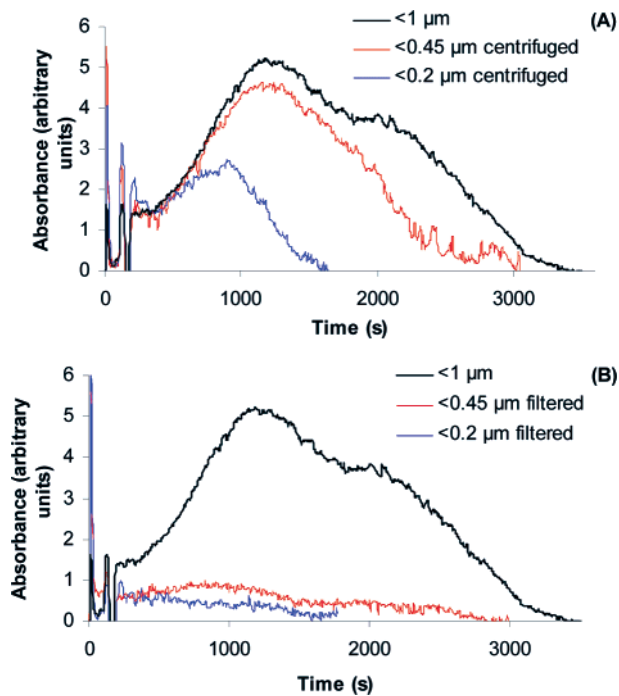


FIGURE 2. SdFFF fractograms for the soil samples comparing filtered and centrifuged fractions with $<1 \mu\text{m}$ starting material: (A) fractogram for <0.2 and $<0.45 \mu\text{m}$ centrifuged fractions with data averaged for the two runs; (B) fractogram for <0.2 and $<0.45 \mu\text{m}$ filtered fractions with data averaged for the two runs.

t_1 and t_a were determined using a computer program written by P. S. Williams (University of Utah, Salt Lake City, UT).

The filtered and centrifuged samples ($80 \mu\text{L}$), and the 1% (m/v) soil suspension ($80 \mu\text{L}$), were injected through a rubber septum into the channel. After a few seconds, the carrier solution was switched to bypass the channel and flowed directly through the detector (relaxation/stopflow). After a 10 min relaxation time at a rotation of 1000 rpm (169g), the channel flow was restored and the run commenced. The initial field of 1000 rpm was held for a time lag, t_1 , of 5.3 min. The decay parameter t_a of -42.0 min then reduced the field to a holding rotation of 20 rpm (0.067g). A DC motor and speed controller (Bodine Electric Co.) powered the rotor.

The absorbance of the eluent was recorded using a Spectra 100 variable wavelength detector (Spectra-Physics, USA) at 254 nm with a sensitivity of 0.02 AUFS. Two runs were carried out for each fraction (<0.2 and $<0.45 \mu\text{m}$ filtered samples; and <0.2 and $<0.45 \mu\text{m}$ centrifuged samples) and the starting material (containing $<1 \mu\text{m}$ particles).

Data Analysis. Fractograms were obtained by plotting detector response against elution volume (or time) of the emerging sample. The fractograms were converted to particle size distributions using an analysis program (Field-Flow Fractionation Research Centre Software, University of Utah, 1990). The fractograms were not corrected for light scattering (30, 32, 39). The negative peak at 2.7 min after the start of each fractogram, resulting from the sample matrix being different from the carrier solution, has been removed from the figures for clarity.

Results and Discussion

Fractograms of Soil Suspensions. The differences in fractograms for the centrifuged and filtered fractions with the $<1 \mu\text{m}$ starting material are shown in Figure 2A and B, respectively. All data are the means of duplicate injections. The UV response for the filtered fractions for both size cutoffs was significantly lower than that for the corresponding centrifuged fractions. Typical reproducibility for duplicate

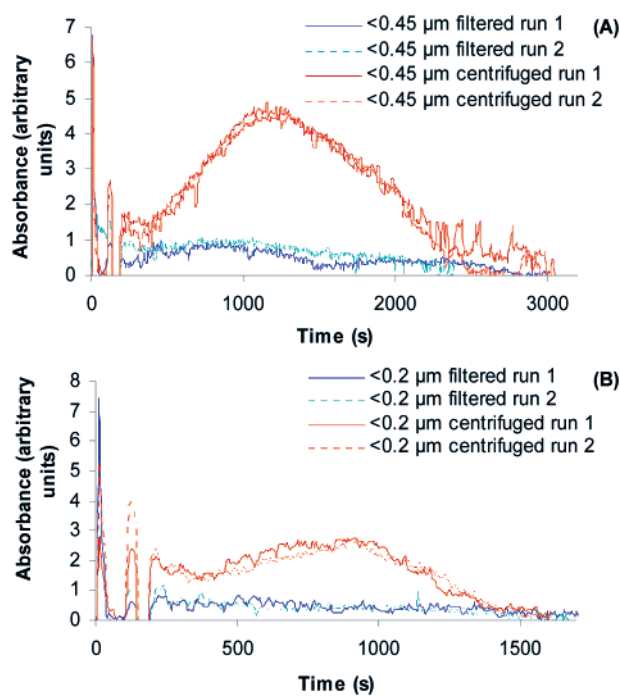


FIGURE 3. SdFFF fractograms for the soil samples showing the good reproducibility observed between two runs: (A) fractograms for $<0.45 \mu\text{m}$ filtered and centrifuged runs; (B) fractograms for $<0.2 \mu\text{m}$ filtered and centrifuged runs.

injections of the centrifuged and filtered fractions is shown in Figure 3A,B for the <0.45 and $<0.2 \mu\text{m}$ runs, respectively.

Particle Size Distributions. The SdFFF instrument was calibrated using polystyrene bead standards of known diameters. The fractograms were converted into particle size distributions (PSDs) and the data for duplicate injections of the starting material, and the <0.45 and the $<0.2 \mu\text{m}$ centrifuged fractions were averaged. These data (Figure 4A) showed that the $<1 \mu\text{m}$ soil sample had a log-normal distribution of particle sizes with a maximum at $0.13 \mu\text{m}$ and an upper threshold at $0.6 \mu\text{m}$. Chen et al. also reported a $0.6 \mu\text{m}$ threshold value for the same Lilydale sample (28). This size threshold was lower than the expected $1 \mu\text{m}$ based on the sample preparation method used, but similar findings have been reported for other environmental samples (24, 25, 27, 31, 32, 39). Chittleborough et al. (27) reported a threshold value of $0.4 \mu\text{m}$ for loamy sand samples, and van Berkel et al. (25) reported a threshold of $0.6 \mu\text{m}$ for both soil and suspended river colloids.

The PSDs for the centrifuged <0.45 and $<0.2 \mu\text{m}$ fractions had upper size thresholds of about 0.40 and $0.18 \mu\text{m}$, which are close to the expected cutoffs (Figure 4B and C). However, some material less than these cutoff diameters was also removed by centrifugation. This may be due to the heterogeneity of the particle shapes and the assumption made about soil particle density in the calculations applied to the raw fractograms.

For the filtration experiments, the initial concentration of the soil suspension (1% m/v) was high but not extreme. Twenty-five milliliters of suspension was filtered, corresponding to a 0.25 g loading of soil particles, which would be equivalent to filtering 1 L of a 250 mg L^{-1} suspension. This is a realistic experimental design. The fact that the soil suspension could be filtered by suction filtration without complete blockage suggests that filter loading was not excessive. Furthermore, the filtration was sequential, and hence the loading on the $0.2 \mu\text{m}$ filter was much less than 0.25 g .

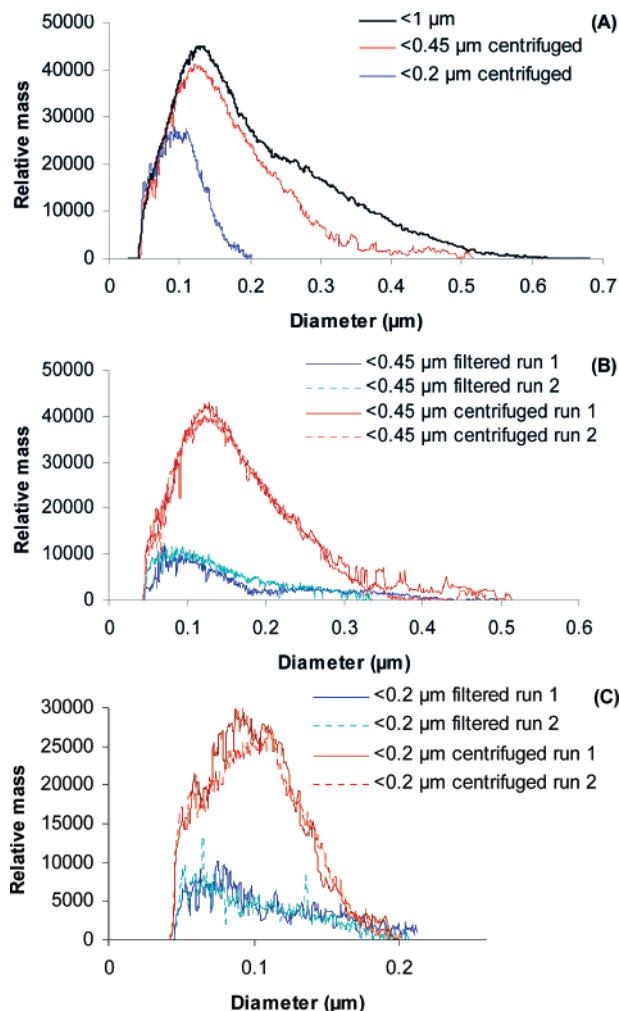


FIGURE 4. SdFFF particle size distributions for the soil samples: (A) particle size distribution for <0.2 and $<0.45\ \mu\text{m}$ centrifuged fractions and $<1\ \mu\text{m}$ starting material with data averaged for two runs; (B) particle size distribution for two runs of $<0.45\ \mu\text{m}$ filtered and centrifuged fractions; (C) particle size distribution for two runs of $<0.2\ \mu\text{m}$ filtered and centrifuged fractions.

The particle size distributions for the filtered <0.45 and $<0.2\ \mu\text{m}$ fractions show the same particle size thresholds, of about 0.40 and $0.18\ \mu\text{m}$, respectively, as the centrifuged fractions (Figure 4B and C). Most important, however, is the observation that the relative mass of the filtered fractions is much lower than the centrifuged fractions. The filtration process would have been more affected by particle shape than the centrifugation process because “platey” particles of smaller equivalent spherical diameter (ESD) would be more effectively removed than spherical or cubic particles for any given nominal filter pore size. However, the effect observed in these results is unlikely to be explained by shape. As an example, if all of the particles in the soil suspension were plates (unlikely) with an aspect ratio of 10:1, then the volume would be 10 times lower than for a cube with the same edge length as the plate dimension. This would result in a decrease in the ESD by a factor of about 2.1, and our results show removal by filtration much lower than this ESD for a given filter. Furthermore, SdFFF gives the ESD irrespective of the shape of the particles. The results therefore suggest that conventional <0.45 and $<0.2\ \mu\text{m}$ membrane filtration techniques for the separation of soil suspensions, and by implication other aquatic matrices, remove much more of the particulate material than the corresponding centrifugation procedure. An added advantage of centrifugation is that it is a less aggressive approach than membrane filtration for

the size fractionation of colloids from environmental matrices.

Practical Applications. For the separation of particles from solution, international water industry “standard” procedures have relied on membrane filtration techniques to operationally define the boundary between “particulate” and “soluble” fractions, with a $0.45\ \mu\text{m}$ nominal pore size membrane being the most commonly used (e.g., ref 40). This is because it is relatively fast and filters out the majority of the biotic and abiotic particles but will, however, not retain some bacteria and colloidal material smaller than $0.45\ \mu\text{m}$ (41). This is an area of much controversy and has been discussed in detail by Haygarth and Sharpley (14). The $0.2\ \mu\text{m}$ nominal pore size membrane is slower but much more efficient at removing the microbial phase (bacteria of 0.2 – $1\ \mu\text{m}$), as well as algae ($>1\ \mu\text{m}$) (42), but smaller unicellular bacteria will still not be retained.

Many of the key studies that have helped to define these boundaries have relied on membrane and ultrafiltration for separation (15, 16), but there are uncertainties that surround these techniques. Colloidal material can interact with the membrane, and the increased concentrations of the retained particles at the membrane surface appear to result in the aggregation of smaller colloids (17). Del Castilho et al. (22) suggested that membrane filtration was preferable to centrifugation as it was the easiest method to use. However, in the present study, the centrifugation method was found to be quick and efficient and yielded fractions with upper size cutoffs much closer to the required values than membrane filtration. Additional experiments, although preliminary in nature, suggest that the observations reported in this paper are also found with contrasting soil types, with more dilute soil suspensions (0.5 and 0.25% m/v) and with flow FFF. This finding has serious implications for the many size-based contaminant speciation studies that have relied on filtration for accurate size fractionation of the particles, for example, the operationally defined filterable reactive phosphorus fraction.

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