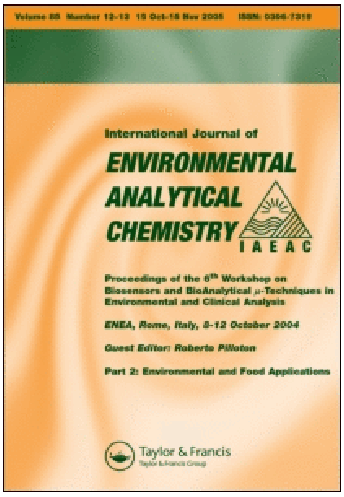

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Sedimentation Field-Flow Fractionation of Colloidal Particles in River Water

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Sedimentation field-flow fractionation (sedimentation FFF) is a new technique that has been used to separate and characterize a variety of submicron particles. With the aid of a newly developed on-channel concentration procedure, sedimentation FFF was used to study the colloidal particles contained in samples collected from the Colorado, Green, and Price Rivers in eastern Utah during the spring run-off season. Samples from the different rivers displayed distinctly different fractograms, which could serve to "fingerprint" and characterize the colloidal content. The fractions obtained, when analyzed, displayed distinct differences in chemical composition. Each fraction re-emerged at its original elution volume when re-injected into the apparatus, indicating that the sedimentation FFF procedure had successfully concentrated then fractionated the colloidal particles in the river water.

KEYWORDS: Field-flow fractionation, water, particulate.

INTRODUCTION

Sedimentation field-flow fractionation (sedimentation FFF) is a relatively new technique that has been applied to the size-based separation and characterization of a variety of submicron particles suspended in liquids. Briefly, particles are sorted out in a flow channel and emerge in a well-defined size (or mass) spectrum. Polystyrene latex beads,[1] viruses,[2] liposomes,[3] protein spheres,[4] and, in unpublished work, emulsions have been among the colloidal materials studied in our laboratory. A recent review of our FFF technology has been published which includes details

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of the sedimentation FFF apparatus and theory.[5] Recently, Kirkland and Yau have applied a unique high speed sedimentation FFF system to various latices, viruses, silica particles, etc.,.[6, 7]

The sedimentation FFF technique has several unique advantages including: (i) the conditions of fractionation are quite mild so that fragile structures can be studied, (ii) the resolution is excellent, (iii) great flexibility is inherent in separations because the external field can be varied readily, (iv) the method is theoretically exact so that particles can be characterized in terms of their behavior in the system, (v) since it is a separation technique based on elution, fractions can easily be collected and examined by other techniques after the size distribution is obtained.

Most sedimentation FFF studies have been limited to materials for which fairly concentrated samples (1 to 10% solids) are available. Recently, a new method of on-channel concentration has been developed for dilute colloidal samples. The method has been tested with two sizes of Dow polystyrene latex beads.[8] In this method, the sample is pumped into the channel at high field strength and low flow rate so that the particles are concentrated in the first 10% or so of the channel. The flow is then stopped and the field reduced. Flow is resumed at a higher rate so that a fractogram—a graph of the relative numbers of particles of a certain effective particle mass versus the elution volume—is obtained.

The study of fine particles in natural waters is important in several respects. Turbidity in water supplies is unacceptable for aesthetic reasons and for its effect on photosynthesis, even when the particles responsible for it are innocuous. Because finely divided solids have relatively large surface areas per unit mass, they can serve as carriers of harmful materials that would otherwise be insoluble, such as heavy metals and lipophilic organic compounds. Phosphorus, a nutrient element important in eutrophication processes, can be transported in natural waters in the form of particles. Using sedimentation FFF followed by the chemical analysis of fractions, it should be possible to identify the particle type acting as the main carrier for these important contaminants.

Many dynamical processes in water are affected by particle size, including sedimentation, aggregation, and particle growth and dissolution. Thus, the knowledge of particle size distribution is important. Some existing techniques (*e.g.* Coulter counters and light scattering) are useful for obtaining size distributions. However, these techniques do not fractionate the sample and they yield incomplete information where the sample is poorly characterized or complex. Microscopy can give good data but yields no fractions and can be time consuming. For the colloidal size range, electron microscopy is required. This can be expensive and, in addition, the particles studied must be immune to the effects of high vacuum and electron beam bombardment. Sample preparation and mounting can become quite complicated for some materials.

With sedimentation FFF, an effective particle mass (or size) distribution is yielded directly in the course of the experimental run. However, for more detailed investigations, the collection of fractions can yield a wealth of information. These fractions can be examined by microscopy or electron microscopy to correlate overall particle morphology with particle size. The chemical analysis of the fractions can then establish a correlation between the content of important elements or compounds and particle size. Since transport rates are strongly influenced by particle size, this procedure could help to establish the transport mechanism and fate of some of the trace metals and organics that strongly affect water quality.

The present work is an exploratory study intended to establish the feasibility of applying sedimentation FFF to natural waters, and it is an attempt to delineate the experimental conditions appropriate to such analysis.

THEORY

A brief review of FFF theory is presented here to aid in interpretation of results. Detailed theory for several FFF techniques[5] and sedimentation FFF in particular[1] has been published.

Retention and separation in FFF are the result of the coupling of a field directed perpendicular to a ribbon-like elution channel and of the differential flow within that channel. With application of the field, particles tend to migrate toward one wall of the channel depending on their buoyancy; they concurrently diffuse away from the channel wall as a result of Brownian motion. They soon reach a steady-state condition, forming a thin layer against the wall of characteristic layer thickness l which is a function of the effective particle mass.

Since the channel is very thin (~0.25 mm) and the flow is laminar, a parabolic flow profile is formed in the channel. The particles of low effective mass interact only weakly with the field, which allows them to diffuse into the higher velocities of the flow profile. Lower mass particles thus elute ahead of higher mass particles which are "trapped" in the low velocity streamlines near the wall (see Figure 1).

Retention on the column is defined by the retention ratio R

$$R = V^0/V_r \tag{1}$$

where V^0 is the void volume, the volume required to elute a non-retained

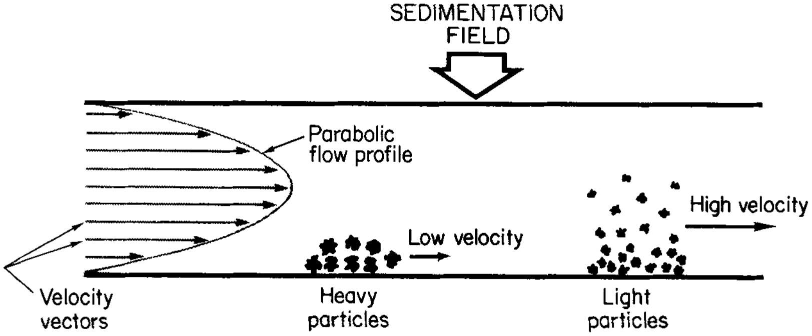

FIGURE 1 Illustration of fractionating mechanism in FFF. The heavy particles are forced closer to the channel wall than the light particles and are thus carried by a slower part of the parabolic flow stream.

component, and V_r is the retention volume of the particle fraction of interest. For well retained components, R can be approximated by[1]

$$R=6\lambda \tag{2}$$

where λ, a basic retention parameter, can be calculated from physical constants, field strength, particle and carrier solution characteristics, and the column dimensions. In the case of sedimentation FFF[9]

$$\lambda=l/w=6kT/\pi d^3 w\Delta\rho G \tag{3}$$

where w is the channel thickness, k is the Boltzmann constant, T is the absolute temperature, d is the equivalent spherical particle diameter, $\Delta\rho$ is the difference between the particle and the carrier solution densities, and G is the magnitude of the sedimentation field expressed as acceleration. By measuring the retention volume V_r of a fraction, Eqs. (1)–(3) can be combined to yield particle diameter d.

In situations where the particle density is not known, the particle effective mass $\pi d^3\Delta\rho/6$ can be determined from the fractogram. Methods for measuring the particle density of monodisperse fractions by sedimentation FFF[10] could perhaps be extended to measure the density distribution of fractions of complex materials.

In the on-column concentration technique, the field applied during sample loading is high enough to minimize sample migration ($R\sim 0$) and the flow rate low enough that the sample will deposit in the first 10% or so of the channel. Equations stating the criteria for sample loading have been obtained elsewhere.[8] When the field intensity G (determined by rotation rate) is reduced, the value of R increases in accordance with Eqs. (2) and (3). The increased R along with the increased flow rate allows the particles to elute fairly rapidly, forming a conventional fractogram.

EXPERIMENTAL

Water samples were collected on June 16, 1980 from the Price River at the crossing with U.S. Highway 50, from the Green River approximately 100 yards upstream of the U.S. 50 bridge at the town of Green River, Utah, and from the Colorado River 3 miles upstream of Moab, Utah, near Arches National Park. Figure 2 shows the sampling locations. Sample preservation consisted of chilling the samples on ice while they were being transported to the laboratory where they were stored at 2°C. The samples were characterized by monitoring the temperature and pH at the time of collection and by performing further chemical and physical tests (see Table I).[11]

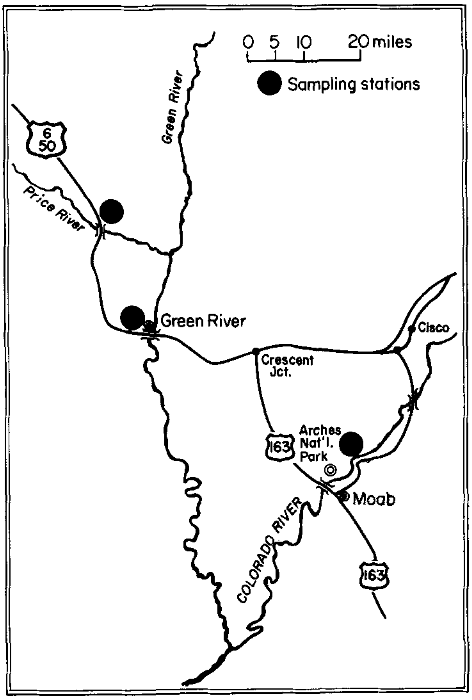

FIGURE 2 Map showing location of sampling sites.

TABLE I

Results of chemical and physical tests for three river water samples. The tests were performed according to "Standard Methods for Examination of Water and Wastewater", 14th Edition (11).

Parameter	Colorado	Green	Price
Alkalinity (mg $CaCO_3$/l)	90	109	286
Conductivity (μmho)	188	170	520
Hardness (mg $CaCO_3$/l)	131	124	371
pH	7.85	8.10	8.35
Residue, Filterable (mg/l)	286	234	710
Residue, Nonfilterable (mg/l)	238	754	1410
Temperature (°C)	16.5	19	18.5

A sedimentation FFF apparatus of the type described previously[1,2,5] was used in the study of the colloidal particles. In the present case the channel dimensions were: length 83.3 cm, width $w = 0.0254$ cm, and breadth 2.0 cm. The void volume was measured to be 4.5 ml. The channel was fitted into an aluminium centrifuge basket with a radius of 7.7 cm. A Gilson Minipuls 2 peristaltic pump was used to pump the carrier solution and the sample to the channel. An Altex model 153 uv detector (operating at 254 nm) was used to detect the particles as they eluted from the channel. A Gilson Microfractionator was used to collect fractions. A Houston Instruments Omniscribe strip chart recorder was used to record the fractograms.

The carrier solution consisted of doubly distilled water with 0.1% by volume FL-70 detergent (Fisher Scientific) and 0.02% by weight sodium azide as a bacteriocide. This solution was degassed prior to use by heating to 70°C.

The water sample was shaken and passed through a loose plug of glass wool in a glass funnel to remove objects such as small twigs. This filtrate was then shaken and allowed to settle for 15 minutes at room temperature in a 100 ml beaker to remove non-colloidal particles. The sample which was studied by means of FFF consisted of 10 ml of the supernatant from this step.

For the concentration step, the sedimentation FFF apparatus was spun at a rate of 1800 rpm (280 gravities) while the sample was pumped into the channel at a rate of 7.5 ml/hr. After the 10 ml sample had been consumed, an additional 3.4 ml of carrier solution was pumped through at the same rate to transfer all the sample from the pump tubes to the channel. The carrier solution flow was stopped for 5 minutes while the field was reduced to either 200 or 900 rpm (3.45 or 70 gravities). The migration-elution step was then initiated by resuming the flow at a rate of 21 to 22 ml/hr while recording the fractogram and collecting 2 ml fractions.

Blanks were run by substituting 10 ml of distilled water and then 10 ml of carrier solution for the sample. A flat baseline was observed in both cases. The reproducibility of the elution volumes was checked by re-injecting selected fractions following the same on-channel concentration and elution procedure as for the original sample.

Some of the fractions were examined by transmission electron microscopy (TEM) using a Phillips EM-201 electron microscope. For this purpose the fractions were deposited directly on formvar grids. Various fractions were also subjected to elemental analysis by an EDAX Model 711 Surface Scanning Microprobe. Here the samples were mounted by dropping the suspensions onto the graphite stub with a Pasteur pipette and evaporating the drop. This process was continued until a visible film was obtained.

RESULTS AND DISCUSSION

Figures 3 to 8 show fractograms obtained for the water samples from each of the rivers at two field strengths. In each case the 200 rpm fractograms show a prominent retained peak corresponding to particles of about 0.2 μm diameter (see below). A shoulder to that peak is apparent for the Colorado and Price but not the Green River fraction. The 200 rpm fractograms also show a smaller peak, corresponding to the larger particles, which appears when the field is turned off.

The 900 rpm runs have a field strength $(4.5)^2 = 20.25$ times greater than that in the 200 rpm runs, leading roughly to 20-fold greater retention volumes for well retained particles. The larger particles are thus shifted far to the right (sometimes emerging only after the field is turned off) and at the same time the finer particles gain enough retention and resolution to display their own characteristic patterns. Most noticeable are the double peaks (the peaks being labeled I and II on the fractograms and in the table) present in all 900 rpm runs, but present in different positions and at different signal heights for the different samples. More work is needed to determine the significance of the two peaks and the origin of their variability.

In general, field strength can be adjusted so that any desired size range can be brought into convenient focus. Alternatively, field programming methods (in which the field strength is varied continuously during the run) make possible the "scanning" of a wider size range in a single run,[12] and can even yield a direct reading of log normal particle size distributions.[7]

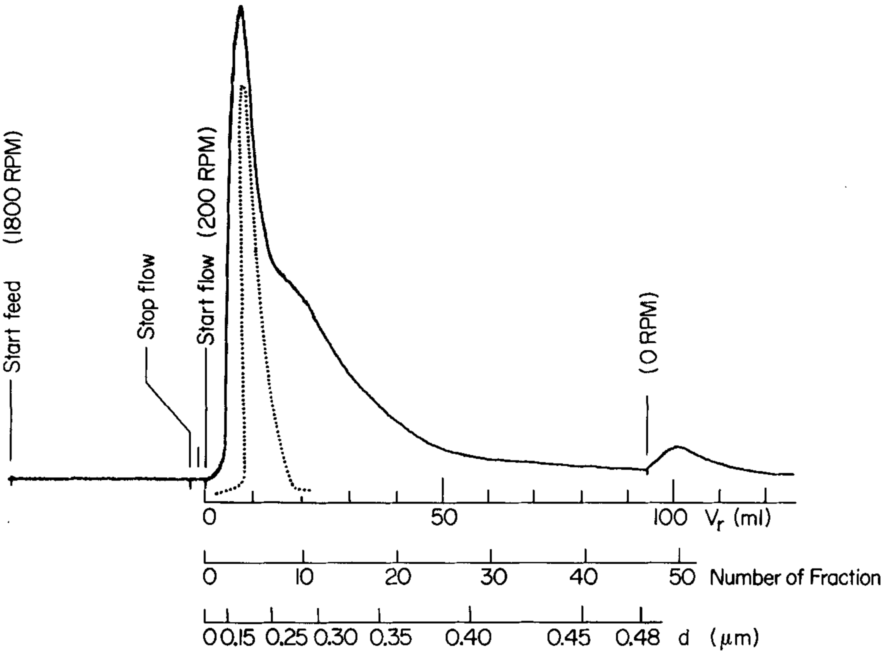

FIGURE 3 Colorado River water fractogram eluted at 200 rpm. Flow rate was 21.4 ml/hr and detector sensitivity was 0.32 at 254 nm.

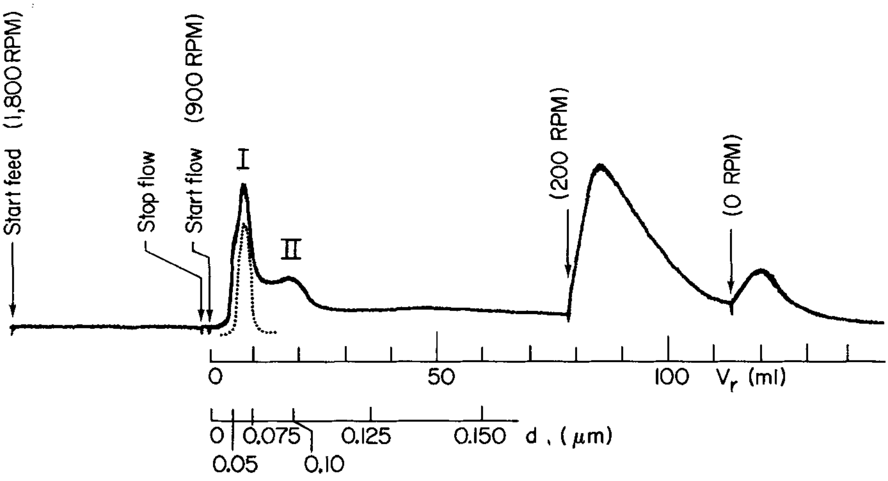

FIGURE 4 Colorado River water fractogram eluted at 900 rpm. Flow rate was 21.8 ml/hr and detector sensitivity was 0.32 at 254 nm.

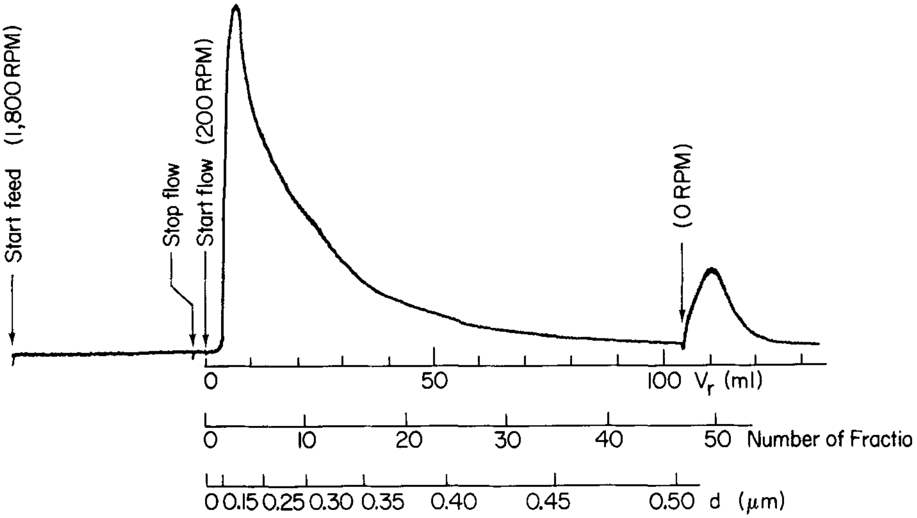

FIGURE 5 Green River water fractogram eluted at 200 rpm. Flow rate was 21.5 ml/hr and detector sensitivity was 0.64 at 254 nm.

Each of the fractograms of Figures 3–8 has a distinctive pattern reflecting the nature of the colloidal content of the water and the FFF operating conditions. With better understanding, such "fingerprints" could undoubtedly be linked to stream flow parameters, pollution, agricultural and rangeland erosion, and other upper watershed conditions. Part of such linkage might be formed empirically by pattern recognition methods. However, FFF and ancillary techniques provide substantial amounts of "hard" data which should greatly improve water characterization. Below, we discuss our preliminary results which are aimed at improving the characterization process.

First of all, we have calculated a particle size scale to go along with the elution volume coordinate in Figures 3–8. The size calculated, the equivalent spherical diameter d shown in Eq. (3), is the diameter of a sphere having the same volume as particles eluting at that point. Our calculations were made assuming a particle density $\rho = 2\,\mathrm{g/cm^3}$ ($\Delta\rho = 1\,\mathrm{g/cm^3}$). While these density values are subject to considerable error, the error in d is much smaller because of its presence in Eq. (3) as the cubed term d^3.

Table II shows the effective mass and d values calculated for the peak maxima for the various samples at 200 and 900 rpm. Note that the two prominent peaks at 900 rpm corresponds to $d \sim 0.1\,\mu m$, as opposed to $d \sim 0.2\,\mu\mathrm{m}$ for the single 200 rpm peaks.

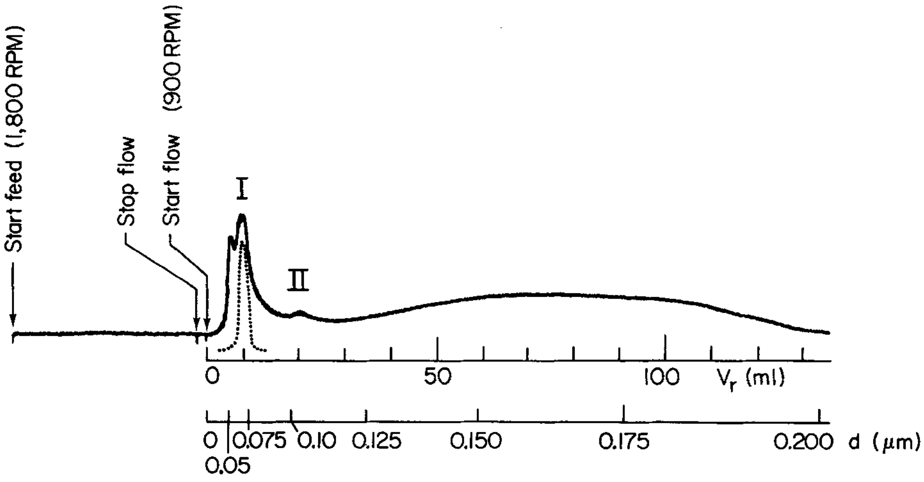

FIGURE 6 Green River water fractogram eluted at 900 rpm. Flow rate was 21.4 ml/hr and detector sensitivity was 0.64 at 254 nm.

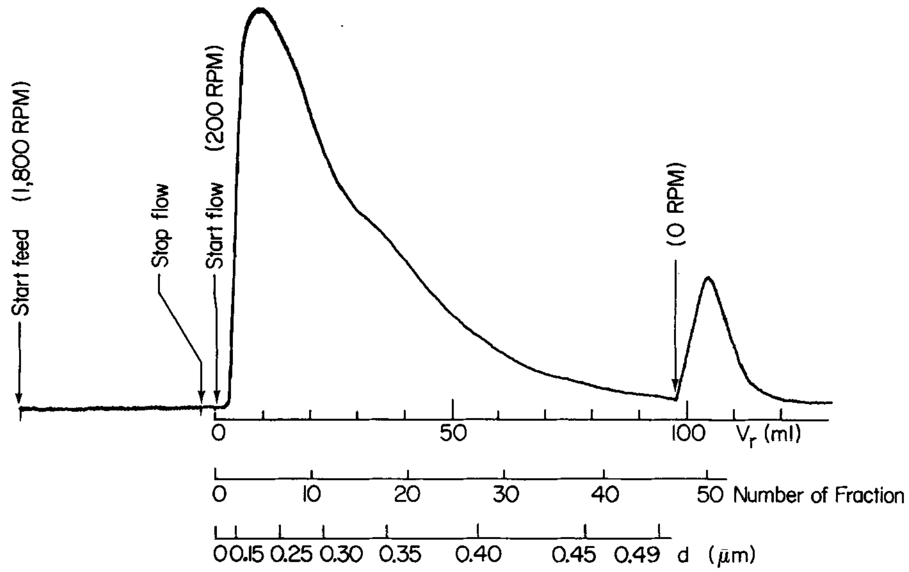

FIGURE 7 Price River water fractogram eluted at 200 rpm. Flow rate was 21.8 ml/hr and detector sensitivity was 0.64 at 254 nm.

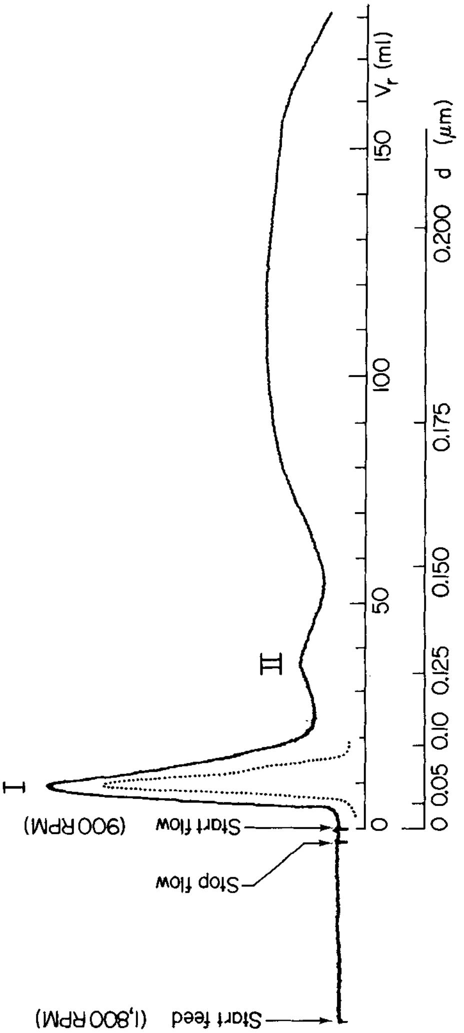

FIGURE 8 Price River water fractogram eluted at 900 rpm. Flow rate was 21.8 ml/hr and detector sensitivity was 0.64 at 254 nm.

TABLE II

Sedimentation FFF characterization of colloidal particles at the peak maximum for FFF fractograms of the various river samples. Effective mass equals true mass less buoyant mass. Peak nos. I and II refer to the first and second peaks, respectively, in the 900 rpm runs

Sample	Field (rpm)	Peak no.	V_r (ml)	λ	Eff. mass (grams)	d* (μm)
Colorado	200		7.00	0.100	4.74×10^{-15}	0.208
Green	200		7.10	0.0986	4.81×10^{-15}	0.209
Price	200		9.33	0.0750	6.33×10^{-15}	0.230
Colorado	900	I	8.05	0.0870	2.69×10^{-16}	0.080
Colorado	900	II	19.20	0.0365	6.43×10^{-16}	0.107
Green	900	I	8.00	0.0875	2.68×10^{-16}	0.080
Green	900	II	20.85	0.0336	6.98×10^{-16}	0.110
Price	900	I	10.10	0.0700	3.34×10^{-16}	0.086
Price	900	II	37.70	0.0186	1.26×10^{-15}	0.134

*Particle density assumed to be 2.0 g/cm^3.

TABLE III

Retention volumes at peak maxima as a function of the age of the samples from the three rivers. Samples were collected on June 16, 1980 (6/16/80) and eluted at the two field strengths shown

			Retention volume (ml)		
	Date	Age	200 rpm	900 rpm	
Sample	of run	(days)		Peak I	Peak II
Colorado	6/25/80	9	7.00		
Colorado	6/30/80	14	6.95		
Colorado	7/7/80	21	7.05		
Fraction	7/9/80	23	7.00		
Colorado	7/11/80	25		8.00	19.10
Colorado	7/14/80	28		8.10	19.30
Fraction	7/16/80	30		8.10	
Green	6/24/80	8	7.10		
Green	6/27/80	11	7.10		
Green	7/2/80	16	7.10		
Green	7/16/80	30		8.00	20.70
Green	7/18/80	32		8.00	21.00
Fraction	7/18/80	32		8.00	
Price	6/26/80	10	9.30		
Price	7/1/80	15	9.40		
Price	7/3/80	17	9.30		
Price	7/23/80	37		10.10	37.70
Fraction	7/25/80	39		10.10	

In order to test the stability of the samples against changes during sample storage, we have compiled Table III which lists the retention volumes for the peak maximum of the different fractograms obtained at various times after sample collection. It appears that these samples were quite stable over the time period of the study.

We have done a separate set of experiments to verify that fractionation actually occurs in the FFF runs. The dotted lines in Figures 3, 4, 6, and 8 show the fractogram obtained when the fraction corresponding to the prominent peak maximum was re-injected and run under the same experimental conditions. The general coincidence in the position of the original peak and the recycled peak, along with the narrowness of the latter, shows that fractionation was accomplished effectively.

An effort was made to confirm the fractionation by electron microscopy. Unfortunately, the sample mounting procedure appeared to aggregate the particles into amorphous clusters of around 1 μm. Consequently, no quantitative data on particle size was obtained from this source.

The elemental analysis of the fractions, by contrast, revealed some clear qualitative trends. Table IV lists the EDAX results for the 200 rpm

TABLE IV

Results of EDAX analyses of river water samples and fractions from sedimentation FFF. Data are in relative percent by weight of elements detected. Fraction numbers in the first column are located by the fraction number scale in figures 3, 5 and 7

Sample	Al	Ca	Cl	Fe	Mg	Na	Si	S	Ti
Colorado									
Orig.	5.540	16.550	4.295	1.464	14.150	33.276	17.465	7.254	—
Orig.	3.841	16.609	4.083	0.622	13.336	38.159	16.564	6.783	—
#4	6.109	1.047	1.215	2.667	—	59.930	28.888	0.141	—
#9	3.485	0.574	0.106	1.020	—	59.199	35.354	0.259	—
#21	1.704	0.434	0.741	1.056	—	62.492	32.949	0.621	—
#51	1.761	0.196	0.395	2.122	—	64.575	30.752	0.196	—
Green									
Orig.	11.898	8.983	2.068	2.169	9.250	28.003	31.691	5.936	—
#4	4.586	0.961	1.575	0.515	—	65.174	27.113	0.073	—
#10	3.378	0.879	1.615	0.903	—	70.151	22.966	0.105	—
#21	3.284	0.395	0.697	0.848	—	71.731	22.904	0.137	—
#51	3.211	0.488	0.782	1.214	—	69.020	25.217	0.064	—
Price									
Orig.	9.612	14.577	0.849	1.658	—	32.011	24.597	16.586	0.106
#5	3.048	1.045	0.587	1.557	—	62.536	30.271	0.935	—
#16	8.502	0.833	0.448	0.792	—	50.244	38.476	0.703	—
#24	2.505	0.879	0.847	0.717	—	62.820	31.788	0.441	—
#50	6.851	1.113	1.341	4.599	—	55.487	30.263	0.303	0.040

fractions and the original samples. Replicate analyses were performed for the Colorado River original sample to check reproducibility, which appeared to be reasonably good. The increase in sodium content observed in the fractions relative to the original samples is attributed to the presence of sodium azide in the carrier solution. The large reduction in the calcium, magnesium and sulfur contents of the fractions indicates that they were present as soluble species which would not have been retained in the concentration procedure. Since the total hardness exceeded the alkalinity (see Table I) for all three rivers, the soluble salts could have included calcium and magnesium sulfate. When the sodium is disregarded, the major elements detected appear to be silicon and aluminium with smaller quantities of calcium, chlorine and iron. This suggests that the colloids consisted mostly of clay or allophane particles. The origin and stability of the samples also suggests that the colloids were largely inorganic rather than organic in nature.

The ratios of the elements clearly vary over the sequence of fractions, suggesting that the sedimentation FFF procedure was fractionating different minerals in different size ranges. While the significance of these trends has not been determined, the attempt to correlate chemical content with particle size has clearly been successful. This provides an important (but not exhaustive) illustration of the type of detailed information obtainable when FFF systems are employed in conjunction with other instruments.

CONCLUSIONS

Sedimentation FFF has been shown to be applicable to a totally new area of analysis where the particle system is complex and the particles are present in much lower concentrations than samples studied previously. The ability of the method to provide sized fractions which maintain their integrity should be invaluable in the detailed study of complex particle systems. Applications to many other complex environmental materials is likely to prove useful.

Acknowledgements

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