

Multi-Wavelength Particle Sizing and Chemical Composition Analysis by Capillary Hydrodynamic Fractionation

Capillary Hydrodynamic Fractionation, CHDF, measures high-resolution particle size distribution (PSD) data in the range 5 nm to 3 microns by separating particles according to size as they are carried by a flowing liquid (eluent) in a capillary tube. The particles are fractionated independently of their density (specific gravity) and exit the capillary in order of decreasing size due mainly to a size exclusion effect and are then carried through a detector, typically an HPLC UV detector operating at either 220 or 254 nm wavelength. The UV-detector output is generated by the sum of absorbed and scattered UV light by the fractionated particles¹.

Fig. 1 below shows a separation obtained under eight minutes of six polystyrene monodisperse standards with sizes 50, 150, 320, 440, 600, and 802 nm. Such separations can be further enhanced by using different capillary ID's or carrier fluid compositions². The surfactant peak originates from molecular species contained mainly in the 50 nm standard³. The molecular peak exits last because its "particle size" is smaller.

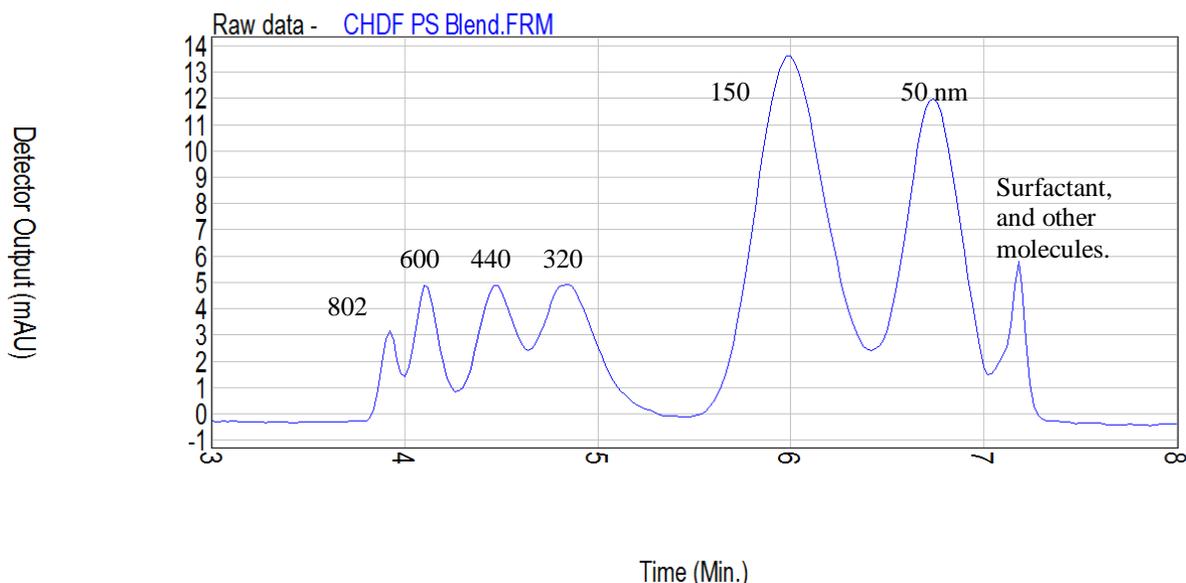
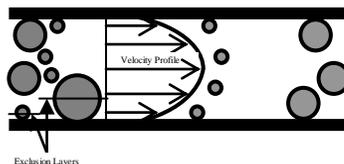


Fig. 1. CHDF Fractogram at 220 nm wavelength of a blend of 6 polystyrene calibration standards.

CHDF calculates PSD data from the particles' transit (elution) time in the capillary tube. Several known-size calibration standards are run in order to create a calibration curve relating a Separation Factor, R_f , versus Particle Diameter. R_f is calculated as the ratio of the elution time of a Marker or internal standard –typically a solution of sodium



benzoate- to that of the particle size standards –usually monodisperse polystyrene beads. Rf increases with particle size and can normally have values between 1 and 2.

CHDF fractionations are independent of particle density. This feature is very useful when analyzing particles whose density or exact chemical composition is not known. This is also advantageous for multi-component samples such as copolymer latexes whereby particle composition –and thus density- may not be uniform throughout the sample.

The remainder of this document discusses how CHDF analysis can be significantly enhanced by multi-wavelength particle detection. Extensive CHDF multi-wavelength detection work has been separately performed by Jai Venkatesan at Lehigh University⁴.

Multi-Wavelength Detection

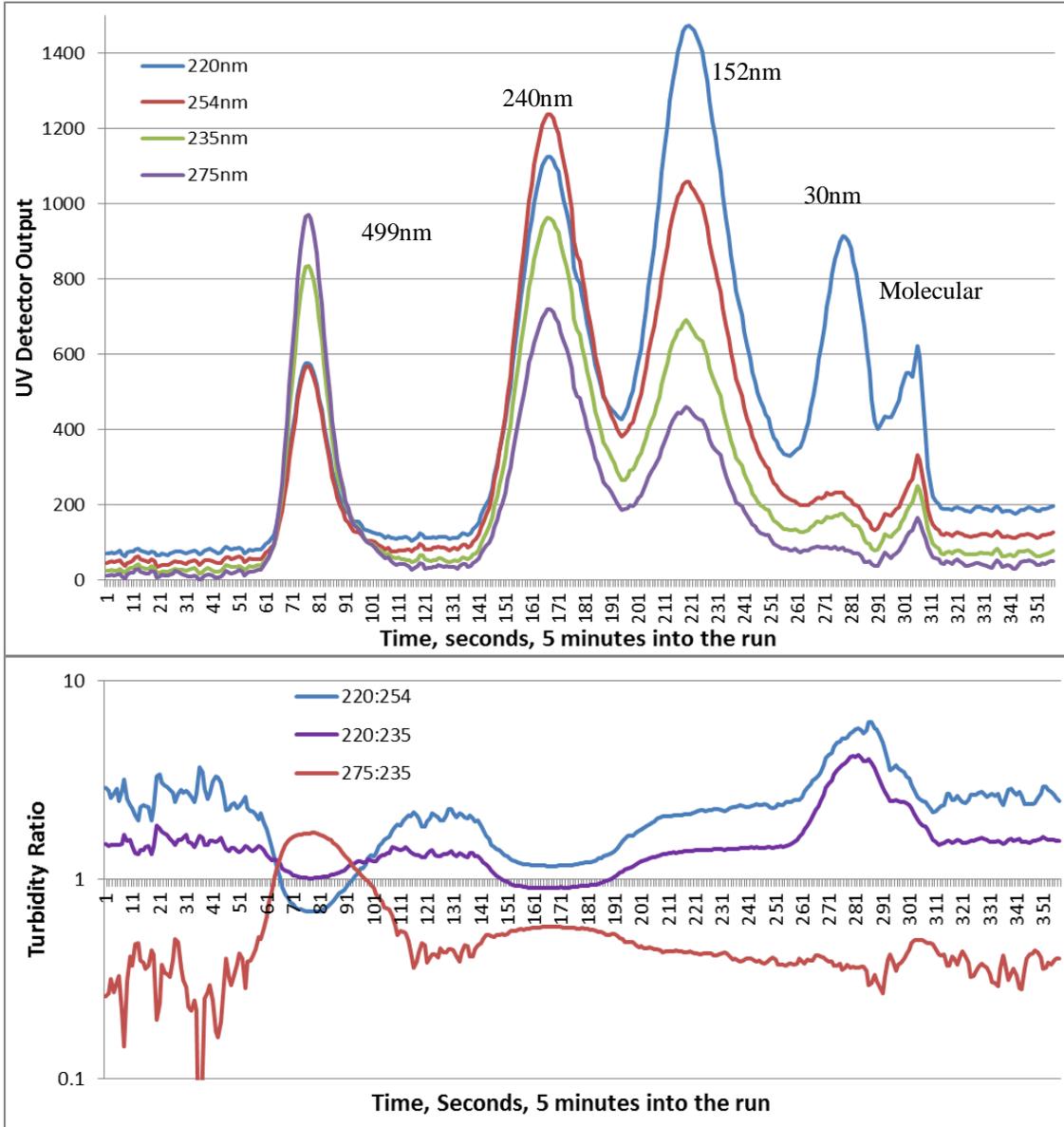
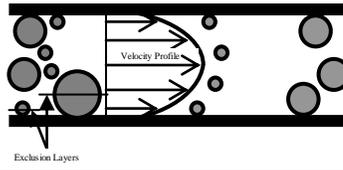
The CHDF fractionation data shown below is from a blend of four polystyrene latex standards of sizes 30, 152, 240, and 499 nm. The time scale corresponds to 5 minutes after sample injection. The four simultaneous fractograms were produced by replacing the CHDF's variable-wavelength detector with a Photo Diode Array (PDA) detector that allows simultaneous detection at four different wavelengths. The PDA detector was operated at 220, 235, 254, and 275 nm wavelengths (not to be confused with particle size values).

This data was automatically produced by a CHDF3000 instrument equipped with an autosampler that allows automatic and unattended particle size distribution analysis by the CHDF3000 software. The CHDF3000 software reads the PDA output for each wavelength once a second and displays the fractograms.

As expected, UV-detection response varies with particle size and wavelength. The 30 nm particles produce stronger UV output at the 220nm wavelength due to enhanced UV absorbance. Conversely, the 499 nm standard generates a taller peak at 275 nm wavelength from dominant UV scattering.

Figure 3 presents a plot of Turbidity Ratio vs. Time. The turbidity ratios are calculated by dividing the response at one wavelength by that at another wavelength, in this case 220:254 nm, 220:235 nm, and 275:235 nm.

This multi-wavelength dependence has been shown to be useful for absolute particle size measurement, without the need for column calibrations, and also for obtaining information about the chemical composition of the particles. While the turbidity ratios can be used to calculate absolute particle sizes, they can also provide particle chemical composition information if the particle size is known from the regular CHDF fractionation^{5,6}.



Figs. 2, and 3. PDA Fractograms (above) and Turbidity ratios (below) for a blend of four polystyrene calibration standards.

Figure 4 presents the turbidity ratios (TR) as a function of particle size from the data in Fig. 2. Each ratio was calculated by taking the UV output at the fractogram peak value for each calibration standard.

These TR curves can also be generated theoretically by Mie theory computations using known particle Refractive Index values⁷.

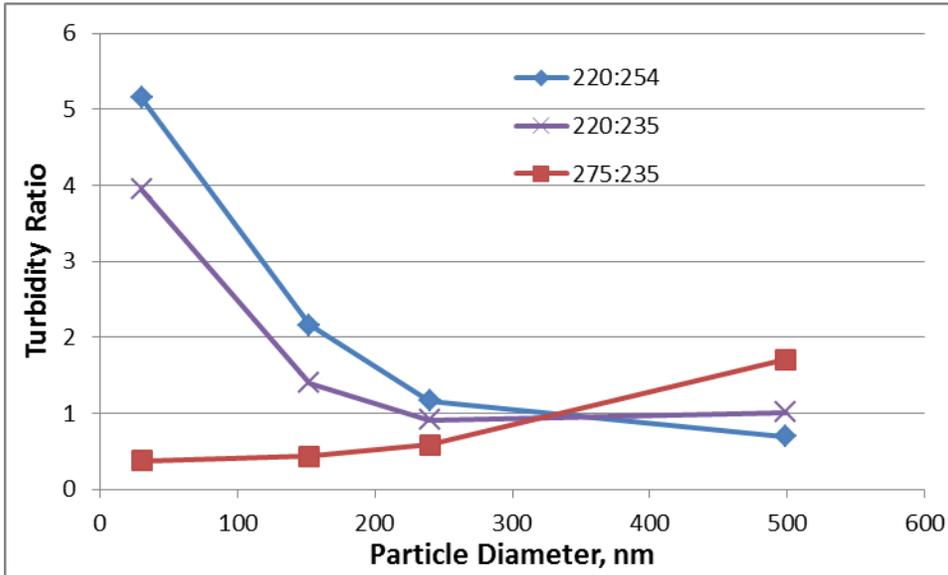
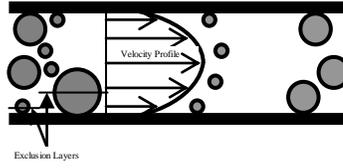
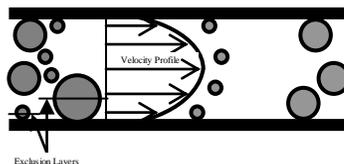


Fig. 4. Turbidity Ratio vs. Particle Diameter for Polystyrene beads.

The set of curves in Fig. 4 would differ for a material with a different Refractive Index. For example, this Refractive Index dependence could be employed to determine the Polystyrene-Butadiene ratio in CHDF-fractionated copolymer latex particles. Additionally, particle coatings can be characterized from their effect on the particle turbidity ratio as exemplified below.

- A researcher expects a certain particle composition. Another researcher is working particle surface coating. Particle refractive index value is a function of particle composition and surface coatings.
- The CHDF software generates theoretical Mie-Theory TR curves from the expected particle refractive index.
- Researcher runs samples on calibrated CHDF column as usual.
- CHDF software compares measured TR curves to Mie-calculated ones. Chemical composition and/or surface properties have changed if Mie and Measured TR curves differ.
- One simple example is a copolymer latex such as Polystyrene-Butadiene (PSB). Polystyrene (PS) and Polybutadiene (PB) separately have their own Mie TR curves. Researcher runs copolymer samples and the CHDF software compares measured TR curves to theoretical (Mie) ones for each particle size data point. Those that match PS, indicate such particles are composed only (or almost entirely) of PS. The same applies to PB curves. Curves in-between indicate those particles contain both polymers. Those TR curves closer to PS indicate a greater PS content. Similarly, one could calculate a PS/PB ratio from the corresponding TR curves.



- Likewise, a particle coating will modify the particle's refractive index (thus, its TR curves). Uncoated particles will produce TR curves matching the particle's pure material.

In summary, absolute CHDF particle sizing can be obtained from TR data. Also, chemical composition and surface property analyses are achieved by running samples on a calibrated CHDF Fractionation column, then using the TR curves which are characteristic for each particle material. Reference TR curves can be produced experimentally, as well as, computationally from Mie Theory.

REFERENCES

1. DiMarzio, E. A. and Guttman C.M., *Macromolecules*, 3, 131, 1970.
2. Dos Ramos, J. G., and Silebi, C. A., *J. Colloid Interface Sci.*, 135, 1, 1990.
3. Dos Ramos, J. G., *Polym. Mat. Sci. Eng.*, 75, 90, 1996.
4. Venkatesan, J., Ph.D. dissertation, Lehigh U., 1993.
5. Venkatesan, J., and Silebi C. A., in *Particle Size Distribution III, Assessment and Characterization*, Provder T. Ed., p. 266, 1996.
6. Bacon, C. P., and Garcia-Rubio, L. H., *Proc. SPIE 3250*, Optical Biopsy II, 110, 1998.
7. Bohren, C. F., and Huffman, D. R., *Absorption and Scattering of Light by Small Particles*, Wiley-Interscience, 1983.