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USE OF AN EVAPORATIVE LIGHT-SCATTERING MASS DETECTOR IN SEDIMENTATION FIELD FLOW FRACTIONATION

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SUMMARY

An evaporative light-scattering detector is valuable in particle-size analysis and molecular-weight determination by sedimentation field flow fractionation. This detection method is particularly advantageous with samples containing particles with diameters smaller than 0.2 μm and with molecules which do not absorb radiation at wavelengths accessible to common spectrophotometric detectors. It overcomes some of the problems inherent in the use of detectors designed for absorption measurements with samples that scatter light and can provide quantitative results on materials without requiring knowledge of their optical properties.

INTRODUCTION

Sedimentation field flow fractionation (SFFF) is useful in molecular-weight determination and particle-size analysis for a wide range of materials[1–4]. The conventional detector for SFFF is a spectrophotometer of the type used in liquid chromatography. Such detectors are designed to measure the absorption of radiation by solutions and are often unsatisfactory for measuring the extinction of turbid systems. Accurate measurement of extinction by a turbid system, such as a colloidal dispersion, requires the rigorous exclusion of radiation scattered at small angles in the forward direction from the detector[5]. There is no such requirement for the detection of absorption by solutions. Most spectrophotometric devices make poor extinction detectors because they do not exclude forward scattered light.

To derive concentrations from turbidimetric signals requires that the refractive index of the sample be known. This is often difficult to obtain, particularly for absorbing samples. In addition, the extinction efficiency of the scatterers, which determines the sensitivity of the detector, is strongly dependent on particle size in the submicrometre range[6,7]. Difficulties are often encountered when data for broad size distributions are analyzed, since the sensitivity to the smallest particles is often one to two orders of magnitude smaller than for the largest particles, which makes quantitation of the small particles difficult.

To overcome some of these problems, Compton *et al.*[8] have described an optical detector which counts individual particles as they elute from a steric field flow

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fractionation system. Although their detector was designed to operate with 1–70-μm particles, detectors using flow ultramicroscopy with intensity measurement have been built for use below 1 μm[9,10]. It should be possible to use such instruments with pulse counting on particles as small as 0.1 μm. These counting devices require no knowledge of the optical properties of the particles and may be of great value for use with dispersions of materials in the appropriate size range.

Another approach to detection recently introduced into the chromatographic field and which is applicable to SFFF is evaporative light-scattering mass detection[11,12]. In such a device the eluent is nebulized by a high-velocity gas stream to produce an aerosol with a broad size distribution which will contain a constant number of particles per unit volume of gas if the eluent and gas flow-rates are constant. The aerosol then passes through a heater which evaporates the eluent and leaves an aerosol of the presumably nonvolatile sample. The size of these particles will depend on the concentration of material in the eluent, provided that the particles in the eluent are smaller than the nebulized droplets. Finally, the aerosol passes through a chamber which is traversed by a light beam, and the intensity of light scattered at some angle is recorded. The intensity of the scattered light may then be related to the concentration of material in the eluent. The use of such a device as a detector in SFFF is described here.

INSTRUMENTATION

The SFFF system was produced by the Clinical & Instrument Systems Division of the DuPont Co. (Wilmington, DE, U.S.A.). It was designed around a Sorvall RC-5B centrifuge and is similar to systems described in the literature[13,14]. The rotor had a maximum speed of 17,500 rpm, and the channel (continuous ring type) had a radius of 9.52 cm, resulting in a maximum force of 32,600 *g*. The channel width was 0.0254 cm. A DuPont Instruments UV spectrophotometer was used for optical detection. The system was used in the time-delayed exponential-decay programmed field mode[13,15]. Control and data collection functions were performed by an HP-9826 computer (Hewlett-Packard, Palo Alto, CA, U.S.A.).

The evaporative light-scattering mass detector was manufactured by Applied Chromatography Systems (Bedfordshire, U.K.). We have recently described the characteristics of this instrument[16]. The mass detector was connected to the SFFF system in series after the optical detector. The stability of the signal from this instrument is very sensitive to changes in the evaporator temperature. After a sudden change in the solvent flow-rate, substantial time is needed for thermal equilibrium to be established. This effect is particularly pronounced for solvents with high heats of vaporization, such as water. Thus it was necessary to maintain flow through the detector when the SFFF system was operating at flow-rates other than that used for elution, such as during sample introduction and relaxation and between runs. An eluent pump separate from the SFFF system was used for this purpose. A Rheodyne 7120 injector was used as a diverter so that the output from the auxiliary pump was directed through the mass detector before elution began. When elution began, the valve was changed so that the eluent from the SFFF system was directed through the mass detector. The evaporator temperature was kept at 86°C, and the nebulizer air pressure was 30 p.s.i. The eluent flow-rate was 2 ml/min, and the output signal was recorded on a strip chart recorder set for 10 mV full scale.

EXPERIMENTAL

Experiments were performed with aqueous eluents containing 0.001 *M* ammonium hydroxide for the Ludox TM (DuPont) samples and 0.1% FL-70 surfactant (Fischer Scientific, Fair Lawn, NJ, U.S.A.) for the polystyrene latices (Dow Chemical, Midland, MI, U.S.A.) and dextran[17] samples. The initial rotor speed was 10,000 rpm, and the speed decay constant was 4 min for the dextran and 3 min for the Ludox TM and polystyrene latices. The wavelength for optical detection was 350 nm for the latices, 230 nm for the dextran, and 254 nm for Ludox TM.

Samples were diluted to the desired concentration with the eluent, and 50-μl injections were made. Relaxation times of 3 to 5 min were used.

DATA REDUCTION

The detector output, in the form of graphs of signal *versus* time, was digitized by use of a Hewlett-Packard HP-85 computer and Summagraphics (Fairfield, CT, U.S.A.) Bit Pad One. The time axis was converted to particle size by use of published equations[13,15]. For optical detection with particulate samples, the signal at each digitized point (S) was divided by the extinction efficiency (Q_{ext}) calculated for particles of the given size (a) and refractive index to yield a mass distribution:

$$S/Q_{ext} = a^3(dN/da) \quad (1)$$

For a high-molecular-weight solution polymer such as dextran, calculation of the scattering efficiency requires a knowledge of the conformation of the molecules. Since this is often not available, the approximation that the sample behaves as a collection of Rayleigh scatterers is sometimes made. In this case the signal divided by the molecular weight yields a mass distribution. This follows from eqn. 1, using the relationships that the molecular weight M is proportional to a^3 and for Rayleigh scatterers Q_{ext} is proportional to a^4 (ref. 6). Of course, such an approximation introduces some uncertainty into the results.

The output of the mass detector was converted into a particle-size distribution by dividing the digitized signal at each point by the particle diameter or into molecular-weight distributions by dividing the signal by the molecular weight. These conversions are necessitated in part by the relationship between elution time and particle size or molecular weight and are valid only for experiments done in the time-delayed exponential-decay mode[18].

Cumulative size distributions were calculated by numerical integration of the differential mass distributions.

RESULTS AND DISCUSSSION

Fig. 1 contains the data traces, signal *versus* time, obtained for an injection of 1.2% Ludox TM using optical (a) and mass (b) detection. The substantially higher signal-to-noise ratio obtained with the mass detector is apparent. The peak at about 1.5 min in each of these traces is due to unretained material in the sample. The relative amount of such material in a sample cannot be obtained simply from the size of this peak because its optical properties are usually unknown.

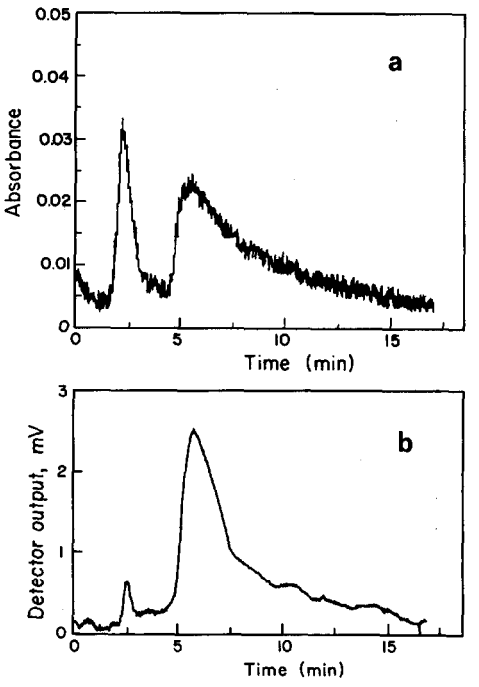

Fig. 1. Data traces for 1.2% Ludox TM from the optical detector (a) and the mass detector (b).

TABLE I

SPECIFIC RESPONSE OF THE EVAPORATIVE LIGHT-SCATTERING MASS DETECTOR AS A FUNCTION OF PARTICLE SIZE FOR LUDOX COLLOIDAL SILICA AND FOR POLYSTYRENE LATICES

Sample	*Diameter* (μm)*	*Response/μg*
Ludox SM	0.0126	4,460
Ludox IBD-1019-69	0.0164	4,930
Ludox HS-40	0.0173	4,790
Ludox AM	0.0182	4,850
Ludox TM	0.0244	4,320
Ludox WP	0.0285	4,290
Latex	0.085	22,600
Latex	0.109	23,300
Latex	0.173	23,200
Latex	0.305	30,700
Latex	0.460	17,200
Latex	0.620	7,800

* The diameters of the Ludox samples were determined by light scattering. The values for the polystyrene latices are those given by the manufacturer.

To examine the effect of sample particle size on the response of the mass detector, we injected samples of Ludox sols and of polystyrene latices directly into the detector and calculated the recorded area per unit mass of sample. The results given in Table I are averages of five to ten determinations on each sample. For the Ludox samples, the standard deviation of the mean is about 6%, and there is no trend to suggest that the variations are other than random. For the polystyrene latices, the instrumental sensitivity is relatively constant for the three smallest samples but is strongly dependent on particle size for samples larger than about 0.2 μm. This observation was not unexpected, since the number size distribution of the liquid aerosol produced by the nebulizer peaks at about 0.14 μm[16]. Therefore, many of the droplets are too small to contain latex particles larger than 0.2 μm, and many fused doublets are likely to be present in the dried aerosol. Such particles may account for the increase in sensitivity observed for the 0.3-μm latex. The scattering intensity per unit mass of sample goes through a maximum as the average particle size of the dried aerosol increases, which explains the decrease in sensitivity for larger particles[6]. The absolute sensitivities found for the Ludox samples should not be compared with those for the polystyrene latices, because the differences in refractive indices of these materials result in different scattering properties.

SFFF experiments were carried out on a series of mixtures of latices, and the mass ratios of the components were calculated from data obtained by both the optical and mass detectors. Fig. 2 contains the mass detector output for the mixture of 0.085- and 0.173-μm latices. The attenuation here is half that used in Fig. 1b. The noise level in the signal is comparable to that observed for water, showing that the 0.1% FL-70 in the eluent contributed little signal. About 90% by weight of FL-70 is volatile at 100°C, which makes this surfactant a good choice for use with the evaporative detector. In contrast, eluent containing 0.1% Aerosol-OT produced a significant background noise level.

Fig. 3 shows the results obtained for this pair of latices by using optical detection both before and after correction for the effects of particle size on scattering power. The optical detector is much more sensitive to the larger particles than to the smaller ones.

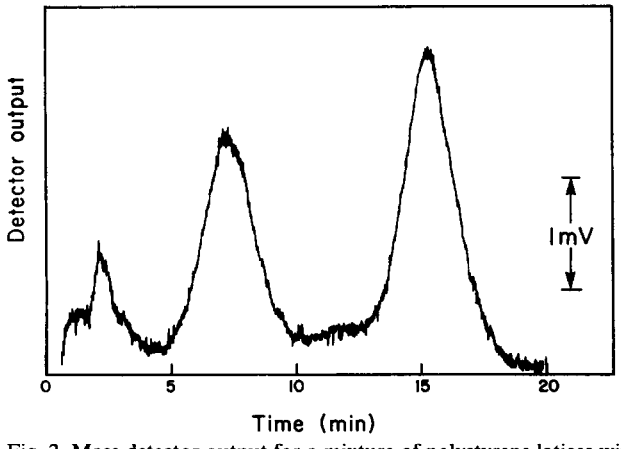

Fig. 2. Mass detector output for a mixture of polystyrene latices with nominal diameters 0.085 and 0.173 μm.

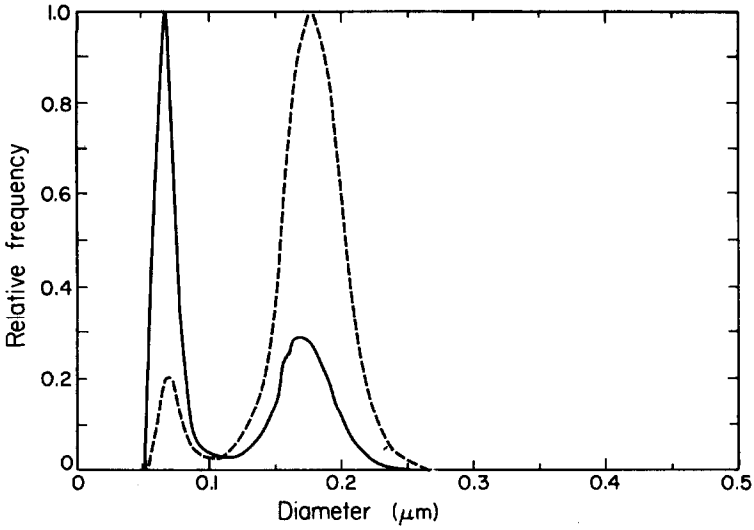

Fig. 3. Mass distribution of particle sizes obtained by optical detection for a mixture of polystyrene laticess with nominal diameters of 0.085 and 0.173 μm before (------) and after (——) correction for the effect of particle size on scattering efficiency.

The results for the mixtures are given in Table II along with the actual mass ratios. As expected in light of the data in Table I, the mass detector gives good results for the two smallest pairs of latices and shows deviations for the larger latices. The optical detector gives erratic results, matching the actual ratios for some pairs and underestimating the fraction of the larger latex in others. Since the optical detector was not designed for turbidimetric measurements, such results are not unexpected.

The detector outputs for the dextran sample are shown in Fig. 4. The signal-to-noise ratio for the mass detector is about three times that for the optical detector. Fig. 5 contains the molecular-weight distributions calculated for dextran by use of the two detection systems. The results are summarized in Table III. The agreement between the SFF/mass detector and light-scattering results is excellent. The

TABLE II

RESULTS OF SFFF EXPERIMENTS ON POLYSTYRENE LATEX PAIRS USING OPTICAL DETECTION AT 350 nm AND EVAPORATIVE LIGHT-SCATTERING MASS DETECTION

*Latex sizes** *(μm)*	*Actual ratio*	*Experimental ratio*	
		Optical detector	*Mass detector*
0.085/0.173	47/53	56/44	45/55
0.109/0.173	59/41	61/39	56/44
0.109/0.305	53/47	68/32	42/58
0.109/0.460	51/49	68/32	57/43
0.305/0.620	33/67	34/66	62/38

* Nominal size from the manufacturer.

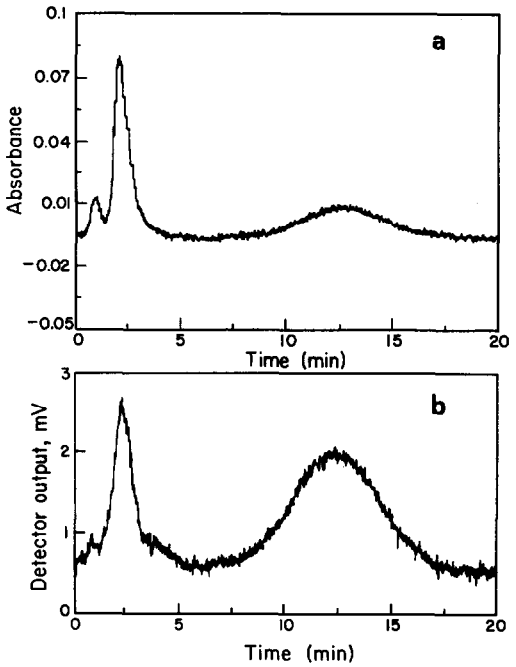

Fig. 4. Data traces for the dextran sample from the optical detector (a) and the mass detector (b).

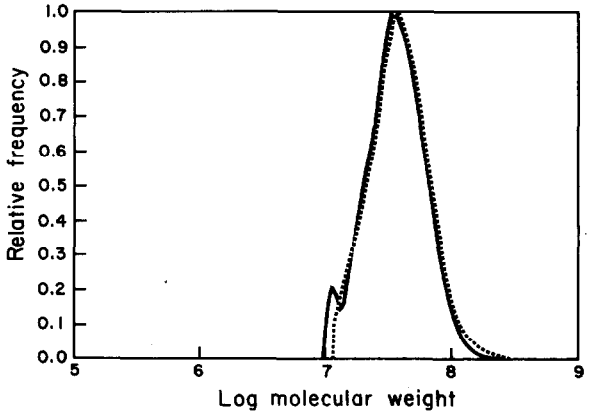

Fig. 5. Molecular-weight distributions calculated for the dextran sample by use of optical detection (------) and mass detection (——).

TABLE III

AVERAGE MOLECULAR WEIGHTS FOR DEXTRAN FOUND BY SFFF AND LIGHT SCATTERING

Method	M_w	M_n
SFFF/optical detection at 230 nm	$6.42 \cdot 10^7$	$4.52 \cdot 10^7$
SFFF/mass detection	$5.76 \cdot 10^7$	$4.30 \cdot 10^7$
Light scattering	$5.88 \cdot 10^7$	

SFFF/optical detection results are somewhat higher than the others, owing to the loss of the signal from the low-molecular-weight end of the distribution into the baseline noise.

CONCLUSIONS

The evaporative light-scattering mass detector, when used with SFFF, can provide accurate molecular-weight distributions and, for particles smaller than 0.2 μm, particle-size distributions. This detector complements particle-counter detectors in that there is little overlap in the size ranges accessible to the two methods. Significant improvements in signal-to-noise ratio, in comparison with turbidimetric detection, can be achieved for samples that have no accessible optical absorption. Uncertainties introduced into the interpretation of turbidimetric or extinction data by a lack of knowledge of the optical properties of the sample are eliminated. For broad size distributions, the large variations in sensitivity of optical detection with particle size, which often makes quantitation of the small size end of these distributions uncertain, is overcome by the use of the evaporative light-scattering detector.

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