Journal of Chromatography, 218 (1981) 217–238
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,093

COMPARISON OF SEDIMENTATION FIELD FLOW FRACTIONATION WITH CHROMATOGRAPHIC METHODS FOR PARTICULATE AND HIGH-MOLECULAR-WEIGHT MACROMOLECULAR CHARACTERIZATIONS

W. W. YAU* and J. J. KIRKLAND
Central Research and Development Department, E. I. Du Pont de Nemours and Company, Experimental Station, Wilmington, DE 19898 (U.S.A.)

SUMMARY

Quantitative particle size and molecular weight determinations by time-delayed exponential force-field sedimentation field flow fractionation (TDE-SFFF) can currently be carried out in 15–20 min using automated apparatus with force fields of up to 50,000 gravities. New resolution parameters provide a common basis for comparing the ability of the commonly used separation methods for particle size analyses. These parameters show that TDE-SFFF has a 5–10 fold and 10–50 fold greater specific resolution than size-exclusion chromatography (SEC) and packed column or capillary hydrodynamic chromatography (HDC), respectively. Because of high resolving power and other characteristics, TDE-SFFF provides superior accuracy in particle size distribution analyses relative to these other separation methods, as confirmed by direct comparisons with typical literature data for a range of particulate samples. TDE-SFFF also has similar advantages over conventional non-chromatographic methods. For example, SFFF exhibits approximately the same resolving power as disc centrifugation but a much wider dynamic range of particle diameter separation in a single analysis.

SFFF provides higher separation resolution than SEC and HDC because of intrinsic differences in retention mechanisms. These latter chromatographic methods separate species by size-exclusion effects —peaks elute prior to the mobile phase solvent —therefore, HDC and SEC are basically limited by available fractionation volume. On the other hand, SFFF exhibits true retention like the affinity liquid chromatography (LC) methods —peaks elute after the unretained mobile phase solvent. In contrast to SEC and HDC, but like LC, TDE-SFFF has the potential for very high peak capacity.

INTRODUCTION

There is growing interest in high-resolution separation methods for characterizing colloidal particles and macromolecular suspensions. Recently, the unique analytical capabilities of sedimentation field flow fractionation (SFFF) have been demonstrated for such materials[1–4]. A new SFFF technique with a time-delayed

exponential force-field decay (TDE-SFFF) has many advantages over constant-field (CF) SFFF for particle size or mass distribution analyses[5–7]. This new approach permits accurate quantitative analyses in the $\leqslant 0.01$–1 μm range in a few minutes.

In addition to TDE-SFFF, other separation methods such as hydrodynamic chromatography (HDC), size-exclusion chromatography (SEC) and disc centrifugation have also been used for characterizing colloidal particles and macromolecular suspensions of $\leqslant 1$ μm. Electron or optical microscopy can be applied to particles in the 0.01–10 μm range, but this approach is tedious and often imprecise. Other separation methods such as the Coulter counter are commonly used for particles of $\geqslant 1$ μm.

Unfortunately, direct comparison of the capabilities of these various methods has not previously been attempted. Such a comparison is needed so that potential users might have the information needed to decide which method would be best for a particular analytical goal. Important features of any particle size analytical method should include the total particle size separation dynamic range achievable in a single experiment, separation resolution or the ability to discriminate between particle sizes, analysis time, convenience and the applicability to various particle types. Unfortunately, two of the most important features, particle size dynamic range and resolution, previously have not been evaluated for the various methods.

The resolution capability inherent in a separation method measures the quality of the separation or the quality of the particle size information obtainable in the optimum size range. High resolution is a prerequisite to accurate and precise particle size analyses[5]. Recently there has been particular interest in methods such as HDC, SEC and disc centrifugation for the <0.1–1 μm particle range. Until now there has been a lack of a common basis for comparing the resolutions of the various particle size analysis methods.

In this paper we propose new specific resolution expressions to serve as a common basis for objectively comparing the resolving power of these most commonly used separation methods for particle analysis. The useful separation range and the dynamic range of some of these methods are also documented. Finally, a descriptive separation performance parameter has been developed that defines the peak capacity of each method. Typical examples of published separations have been utilized to compare the important performance features of some of the separation methods. Comparative data from some conventional non-separation particle size analytical techniques also are included.

COMMONALITY IN RESOLUTION

The extent of separation for two species can be described by the well-known chromatographic resolution expression[8]:

$$R_s = \frac{\Delta V_R}{4\sigma} \tag{1}$$

where, ΔV_R is the difference in peak retention volumes for the individual species and σ is the peak standard deviation in retention volume units resulting from instrumental band broadening. The resolution value R_s provides a measure of the fidelity of the information in terms of the discriminating power (ΔV_R) as modulated by the un-

certainties (σ) in the analytical information. This resolution expression can be applied to many types of analytical information, as well as to chromatographic results. However, the general resolution expression can provide specific information about the resolving power of particle size separation methods only when these resolution values can be normalized in terms of particle size differences. This situation is further complicated by the fact that the dependence of ΔV_R and σ on particle size varies greatly from one particle size analytical method to the other. Thus, the direct use of common R_s values to compare different separation methods is not practical and a more basic resolution parameter is needed.

In previous studies of SEC[9] and SFFF[5] we described the use of the specific resolution factor to provide a general description of resolving power through reduced parameters. In the case of particle size analysis, a variety of particle retention and band broadening mechanisms are involved, and an exact resolution parameter that can serve all separation methods equally well does not appear to be feasible. However, we show here that the specific resolution parameter can satisfactorily describe the capability of many separation methods. Approximations are necessary in this comparison since the specific resolution values are not strictly independent of particle mass M and particle diameter d_p. However, errors due to these approximations appear to be small, and the large differences in the resolution which exist between some methods are clearly demonstrated. Thus the proposed semi-quantitative resolution concepts provide for the first time a reasonable comparison of different separation methods on the same scale.

We have considered the resolution of two general forms of particle size separations. In Fig. 1a, for Case I peak band broadening increases with peak retention and is

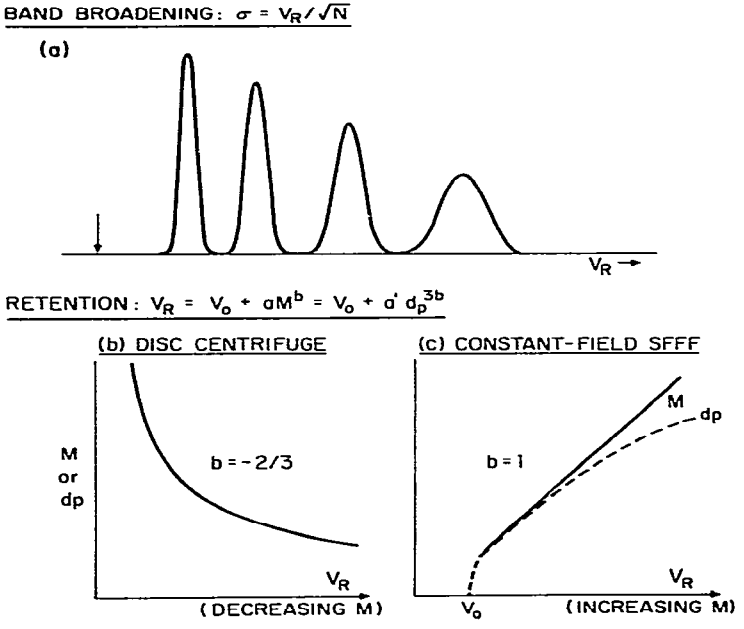

Fig. 1. General forms of particle size separation —Case I.

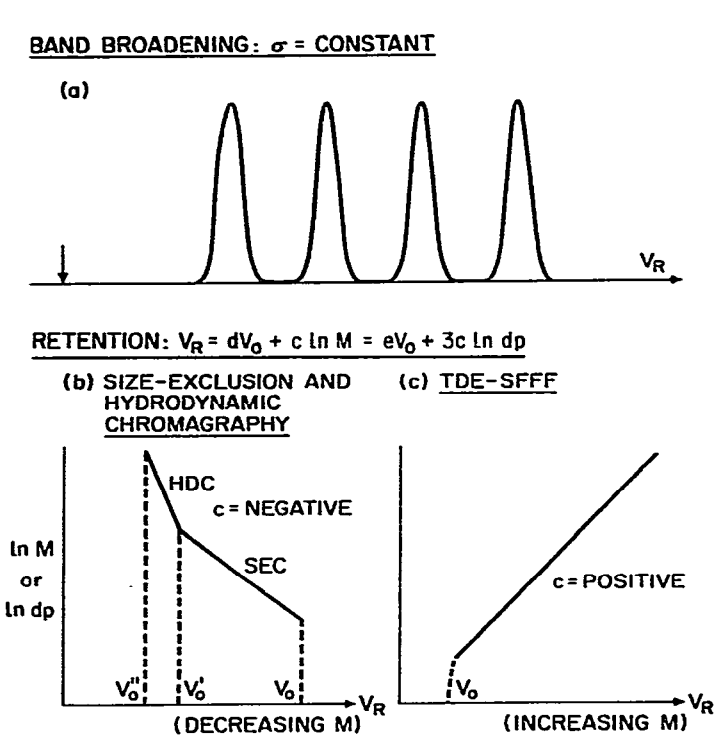

Fig. 2. General forms of particle size separation —Case II.

described by $\sigma = V_R/\sqrt{N}$, where plate count N remains constant. For case II band broadening is independent of peak retention and peaks exhibit a constant σ, as in Fig. 2a. For Cases I and II the dependence of retention volume V_R on particle mass M and particle diameter d_p is considered as:
Case I:

$$V_R = V_0 + aM^b \approx V_0 + a' d_p^{3b} \tag{2}$$

Case II:

$$V_R = dV_0 + c \cdot \ln M = eV_0 + 3c \cdot \ln d_p \tag{3}$$

where, $M \propto d_p^3$ for spherical particles, V_0 is retention volume for an unretained solvent peak, and a, b, c, d, and e are numerical constants. While actual band broadening and particle retention for particular separation methods may deviate somewhat from Cases I and II, there usually is a sufficient resemblance to identify the separation method of interest with one of these cases. For example, the general non-linear retention relationship expressed by eqn. 2 approximately describes disc centrifugation (Fig. 1b), where $V_0 = 0$ and $b = -2/3$, and CF-SFFF with b = 1 (Fig. 1c). Both of these separation methods have peak dispersion characteristics similar to that described by Case I. SEC, both column chromatography and HDC, and TDE-SFFF identify with Case II (Figs. 2b and 2c and eqn. 3). The logarithmic separation depicted

by eqn. 3 describes HDC and SEC with a negative value for the constant c, as illustrated in Fig. 2b. Data from TDE-SFFF demonstrate a positive c value, as depicted in Fig. 2c.

The band broadening and retention characteristics of Cases I and II (Figs. 1 and 2) approximate most of the typical separations commonly encountered in particle size analyses. Therefore, these two cases have been used in this work as a basis for developing a comparison of various particle size analysis methods.

Case I (retention of eqn. 2; constant N)

To develop the resolution relationship for Case I, eqn. 2 is combined with eqn. 1 to yield:

$$R_s = \frac{a\,\Delta M^b}{4V_R/\sqrt{N}} \tag{4}$$

For significantly retained peaks, and small ΔM differences, $V_R \approx aM^b$ and $\Delta M^b \approx (bM^{b-1})\,\Delta M$. Therefore, R_s may be defined in terms of particle mass M as:

$$R_s \approx \frac{(a|\,b|\,M^{b-1})\,\Delta M}{4aM^b/\sqrt{N}} \cong \left(\frac{|\,b|\sqrt{N}}{4}\right)\left(\frac{\Delta M}{M}\right) \tag{5a}$$

or for particle diameter d_p:

$$R_s \approx \left(\frac{|\,b|\sqrt{N}}{4}\right)\left(\frac{3d_p^2\,\Delta d_p}{d_p^3}\right) \cong \left(\frac{3|\,b|\sqrt{N}}{4}\right)\left(\frac{\Delta d_p}{d_p}\right) \tag{5b}$$

where ΔM and Δd_p are the particle mass or diameter differences, respectively, for a pair of species of interest. (Note that the absolute value $|\,b|$ is used in these expressions.)

To provide a general measure of separating power, it is appropriate to define a specific resolution factor, $R_{s,(1+x)}$, which in terms of particle mass M, may be expressed as:

$$R_{s,(1+x)} = xR_s \Big/ \left(\frac{\Delta M}{M}\right) \tag{6a}$$

and for particle diameter d_p:

$$R_{s,(1+x)} = xR_s \Big/ \left(\frac{\Delta d_p}{d_p}\right) \tag{6b}$$

where, x is the fractional particle mass or diameter differences for a pair of species of interest. For example, a particle diameter resolution of $R_{s,1,2} = 1$ describes a separation method that can distinguish a 20% difference in particle diameter with a resolution of unity. This definition of particle resolution will become clearer in later discussions.

The specific resolution parameter is very similar in form to that previously developed for SEC[9]. For the Case I type separations presently under consideration, we can also show for particle mass:

$$R_{s,(1+x)} = \frac{|b|\sqrt{N}x}{4} \tag{7a}$$

and for particle diameter:

$$R_{s,(1+x)} = \frac{3|b|\sqrt{N}x}{4} \tag{7b}$$

It should be noted that eqns. 7a and 7b are approximate expressions that are valid for small particle size differences (small x values). The exact specific resolution expressions derived in the Appendix should be used to calculate specific resolution values for large particle size differences.

Case II (retention of eqn. 3; constant σ)

In a manner equivalent to that described for Case I, specific resolution factors can be developed for Case II for small particle size differences. By combining eqn. 3 with eqn. 1, we can define R_s in terms of particle mass M as:

$$R_s = \frac{\Delta V_R}{4\sigma} \approx \frac{|c|\,\Delta \ln M}{4\sigma} \approx \frac{|c|}{4\sigma}\left(\frac{\Delta M}{M}\right) \tag{8a}$$

and in terms of particle diameter d_p:

$$R_s = \left(\frac{|c|}{4\sigma}\right)\frac{3d_p^2\,\Delta d_p}{d_p^3} \cong \frac{3|c|}{4\sigma}\left(\frac{\Delta d_p}{d_p}\right) \tag{8b}$$

(Note that the absolute value $|c|$ is used in these resolution expressions.) The specific resolution factors $R_{s,(1+x)}$ for Case II can now be defined. For particle mass:

$$R_{s,(1+x)} = \frac{|c|x}{4\sigma} \tag{9a}$$

and for particle diameter:

$$R_{s,(1+x)} = \frac{3|c|x}{4\sigma} \tag{9b}$$

Eqns. 9a and 9b are approximate expressions that are only valid for small particle size differences (small x values). Exact specific resolution relationships are developed in the Appendix.

It is important to note that in eqns. 5 and 8, resolution R_s is directly proportional to $\Delta M/M$ or $\Delta d_p/d_p$, the fractional difference in particle mass or particle diame-

ter, respectively. Because of this unique result, we now can expect the specific resolution $R_{s,(1+x)}$ to compare adequately the resolving power capability of each of the various forms of particle size separations discussed for both cases above. This specific resolution factor generally is used to describe the performance of a particular method, independent of sample polydispersity. However, monodispersed standards typically are used for evaluating the specific resolution values of particular separation methods.

Discrimination capacity

Based on the $R_{s,(1+x)}$ concept, another resolution parameter[10] termed the particle mass or particle diameter discrimination capacity X_M or X_{d_p} is defined. X_M and X_{d_p} values describe the minimum fractional differences in M or in d_p that a particular method can separate with a resolution of unity. Stated otherwise, X_M and X_{d_p} values are simply the required x values to satisfy eqn. 6 by setting $R_{s,(1+x)} = 1$. For example, a X_{d_p} value of 0.2 means that a particular method can discriminate a pair of species with a 20% or larger difference in particle diameter with resolution of unity or more.

The discrimination capacity relationship takes the form:

$$X_M = \left(\frac{\Delta M}{M}\right) \Big/ R_s \tag{10a}$$

and

$$X_{d_p} = \left(\frac{\Delta d_p}{d_p}\right) \Big/ R_s \tag{10b}$$

For Case I separations, eqn. 10 becomes:

$$X_M = \frac{4}{|b|\sqrt{N}} \tag{11a}$$

or

$$X_{d_p} = \frac{4}{3|b|\sqrt{N}} \tag{11b}$$

and for Case II separations:

$$X_M = \frac{4\sigma}{|c|} \tag{12a}$$

or

$$X_{d_p} = \frac{4\sigma}{3|c|} \tag{12b}$$

Peak capacity

Another useful resolution parameter in particle separations is the peak capacity of the method[11]. For particle size or mass separations we propose the parameter $N_{max.}$ to describe the maximum number of fully resolved ($R_s = 1$) peaks that can be obtained within the dynamic range of a separation method. This peak capacity parameter is based on the discrimination capacity X_M or X_{d_p} of the method in relation to the dynamic range DR_M or DR_{d_p} for the same method. Based on the definition of the discrimination capacity (eqns. 10a and 10b), the particle mass or diameter ratio of the resolved species is defined by $(1 + X_M)$ or $(1 + X_{d_p})$. The number of pairs of these resolved species that can fit into the available dynamic range is specified by:

$$(1 + X_M)^{N_{max.}} = DR_M$$

or

$$(1 + X_{d_p})^{N_{max.}} = DR_{d_p} \tag{13a}$$

Then by rearranging:

$$N_{max.} = \frac{\ln DR_M}{\ln (1 + X_M)} = \frac{\ln DR_{d_p}}{\ln (1 + X_{d_p})} \tag{13b}$$

where DR_M and DR_{d_p} are the dynamic ranges of a separation expressed in terms of the ratio of the largest to the smallest particles separable in a single experiment for either particle mass M or particle diameter d_p. For example, for a method having a discrimination capacity X_{d_p} of 0.5 (minimum fractional difference in particle diameter that can be clearly discerned), the particle diameter ratio corresponds to $(1 + X_{d_p}) =$

TABLE I

SPECIFIC PARTICLE DIAMETER RESOLUTION EXPRESSIONS

Method	*Specific resolution**, $R_{s,1+x}$	*Discrimination capacity***, X_{d_p}
CF-SFFF	$\frac{3x\sqrt{N}}{4}$	$\frac{4}{3\sqrt{N}}$
Exponential SFFF	$\frac{3\tau Fx}{4\sigma}$	$\frac{4\sigma}{3\tau F}$
HDC, SEC	$\frac{3x}{4\sigma D_2}$	$\frac{4\sigma D_2}{3}$
Disc centrifugation	$\frac{x\sqrt{N}}{2}$	$\frac{2}{\sqrt{N}}$

* For molecular weight, $R_{s,(1+x)\,(mass)} = 1/3\ R_{s,(1+x)d_p}$.
** For molecular weight, $X_M = 3X_{d_p}$.

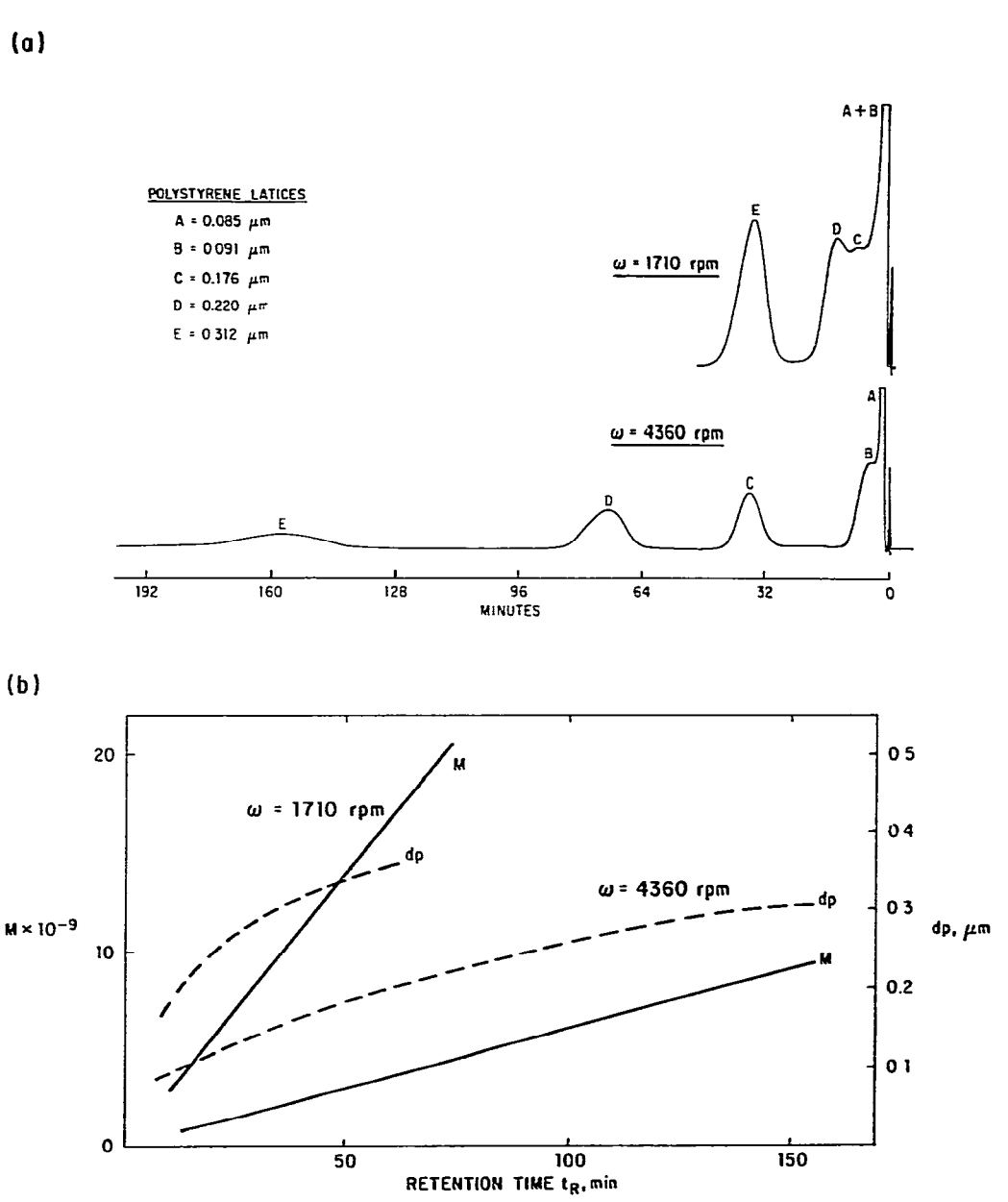

Fig. 3. CF-SFFF data; effect of rotor speed. (a) Fractogram; (b) calibration. Channel, 57 × 2.54 × 0.0254 cm; mobile phase, 0.1% FL-70; flow-rate, 2.0 cm^3/min; relaxation, 10.0 min at ω_0 = 10,000 rpm; sample, 25 μl of 0.1% 0.085 μm, 0.09% 0.091 μm; 0.04% each of 0.176-, 0.220-, 0.312-μm PS latex standards; detector, UV, 300 nm; temperature, 22°C. (Taken from ref. 5.)

TABLE II

PERFORMANCE OF VARIOUS SEPARATION METHODS IN PARTICLE SIZE ANALYSIS

Method	*Reference*	*Polystyrene standards (μm)**	*Dynamic range* (DR_{d_p})	*Specific resolution* ($R_{s,1,2}$)	*Discrimination capacity* (X_{d_p})	*Peak capacity* ($N_{max.}$)
CF-SFFF						
(1710 rpm)	5	0.085– 0.312	2	1.5	0.13	5.7
(4360 rpm)	5	0.085– 0.312	2	1.6–2.3	0.13–0.09	5.7–8.0
TDE-(Exponential)-SFFF						
(3.0 ml/min)	5	0.091– 0.481	6	1.2	0.17	11.4
(1.5 ml/min)	5	0.091– 0.481	6	0.89	0.22	9.0
Disc centrifugation	13	0.5 – 1.01	2	1.2–1.4	0.17–0.14	4.4–5.3
Packed-Column HDC	14	0.038– 0.176	5	0.10	1.9	1.5
Capillary HDC	15	1.0 –10.0	50	0.055	3.7	2.6
SEC	19	0.085– 0.183	3	0.26	0.76	1.9

* Diameter of polystyrene latex standards in representative samples selected for comparison.

1.5. In this case, for a dynamic range $DR_{d_p} = 10$, peak capacity $N_{max.} = 5.7$ as calculated by eqn. 13b ($N_{max.} = \ln 10/\ln 1.5 = 5.7$).

Working equations for specific separation methods

Specific particle diameter resolution expressions for SFFF, HDC, SEC and disc centrifugation are summarized in Table I. These relationships have been based on: $b = 1$ for CF-SFFF, $b = -2/3$ for disc centrifugation, $c = \tau \cdot F$ for TDE-SFFF and $c = -1/D_2$ for SEC and HDC, where τ is SFFF exponential-decay time constant (min)[5], F is the volumetric flow-rate (cm^3/min) and D_2 = molecular weight calibration curve constant (ml^{-1})[12].

RESULTS

Data taken from the literature have been used to develop comparative information in Table II on the dynamic range DR_{d_p}, the specific resolution of $R_{s,1.2}$, the discrimination capacity X_{d_p}, and the peak capacity $N_{max.}$ of several separation methods used for particle size analysis. These comparative values are based on polystyrene latex standards of approximately the same particle size range and separation times of no more than one hour. The highly discriminating $R_{s,1.2}$ value was arbitrarily selected to enable a critical comparison of the high-resolution methods, SFFF and disc centrifugation.

CF-SFFF

The CF-SFFF fractograms shown in Fig. 3a were taken from ref. 5 and analysed in the following manner. At a rotor speed of $\omega = 1710$ rpm the retained 0.312 μm polystyrene (PS) latex peak exhibits a plate number $N = 103$ for the separation. The specific resolution $R_{s,1.2}$ is calculated by eqn. 7b as 1.5 for this run. Since SFFF separations do not exhibit a constant plate number as a function of retention volume[16], the calculated plate number of the higher force field separation at 4360 rpm increases from 112 to 243 then to 313 for PS peaks C, D and E, respectively. This result causes a $R_{s,1.2}$ value increase of 1.59 to 2.34 to 2.65, respectively. The last peak elutes at about 2.5 h and is not tabulated in the Table II summary because it exceeds our arbitrary comparison guideline of limiting separation times to about one hour. The dynamic range of the separations in Fig. 3a is estimated to be about 2 in particle diameter. This means that in a one-hour analysis with CF-SFFF, the range in particle diameter covered by the separation would be no more than two-fold difference in diameter. This is a very narrow dynamic range and is the inherent disadvantage of CF-SFFF for the analysis of samples with wide particle size distributions.

The calibration plot in Fig. 3b shows the expected linear relationship between particle mass (molecular weight) and retention time, and a non-linear (cubic power) dependence on particle diameter[5]. Increase of force field decreases the slope of the calibration plot, leading to an increase in resolution at the expense of reduced dynamic range for the separation.

TDE-(exponential)-SFFF

The TDE-SFFF separations in Fig. 4a (from ref. 5) performed at two different flow-rates were similarly evaluated in terms of performance. The peaks for $F = 3.0$

and 1.5 cm^3/min runs exhibited average σ values of 1.8 and 1.2 ml, respectively (τ = 4.76 min). It should be noted that the results tabulated for TDE-SFFF in Table II are based on a 20-min separation; much higher performance is expected for an one hour analysis. Note also that resolution improves with increasing flow-rate. This result is in keeping with the fact that higher resolution occurs at a higher force field in SFFF. Higher flow-rates in TDE-SFFF cause all particles to elute at a higher force field with resultant higher resolution[5].

Fig. 4b shows the predicted log-linear TDE-SFFF calibration relationship between the logarithm of particle diameter and retention time[5]. As expected, flow-rate variation changes the range of particle separation but has only a small effect on resolution.

Based on the data in Table II, it is apparent that the dynamic range and peak capacity of TDE-SFFF are significantly larger than those of CF-SFFF. For TDE-SFFF, the dynamic range and peak capacity have been improved with only a slight degradation of values for the specific resolution $R_{s,1.2}$ and discrimination capacity X_{d_p}, relative to CF-SFFF. However, on balance, TDE-SFFF is much superior for carrying out particle size analysis. Band broadening corrections are negligible in SFFF and generally are not required in data handling for accurate particle size analyses[5].

Disc centrifugation

The values in Table II for the performance of the disc centrifugation method are calculated from the separation in Fig. 5a (taken from ref. 13). The disc centrifugation method exhibits high resolution but a narrow dynamic range, much like CF-

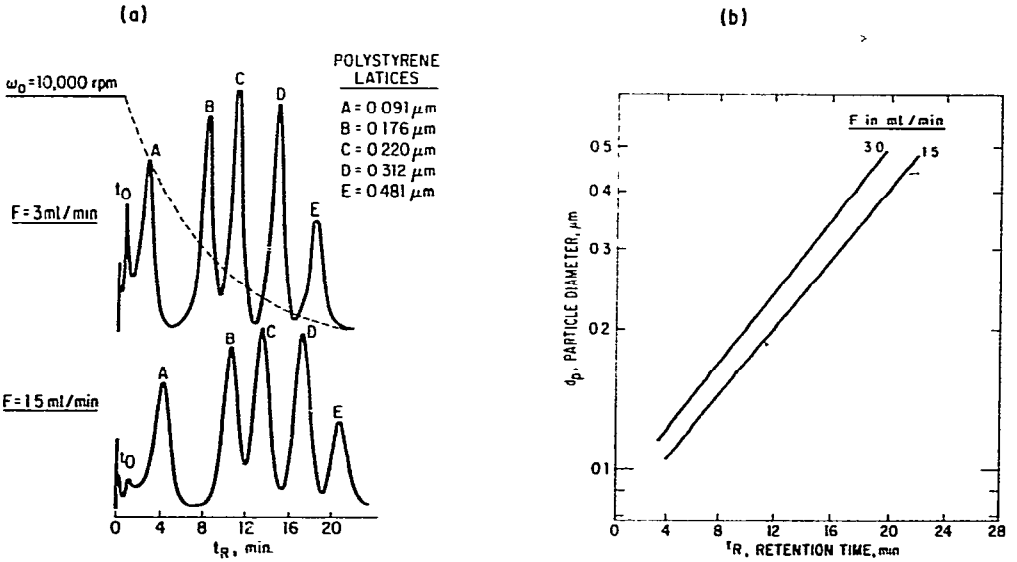

Fig. 4. Exponential field-programmed SFFF data; effect of flow-rate. 10-μl of polystyrene standards: 0.09% 0.091 μm; 0.04% ea. of 0.176-, 0.220- and 0.312-μm; 0.05% 0.481 μm; detector, UV, 254 nm; 0.1% FL-70 mobile phase; channel, 57 × 2.54 × 0.0125 cm; flow-rate, 3.0 and 1.5 cm^3/min; initial rotor speed, 10,000 rpm; decay time constant τ, 4.76 min.

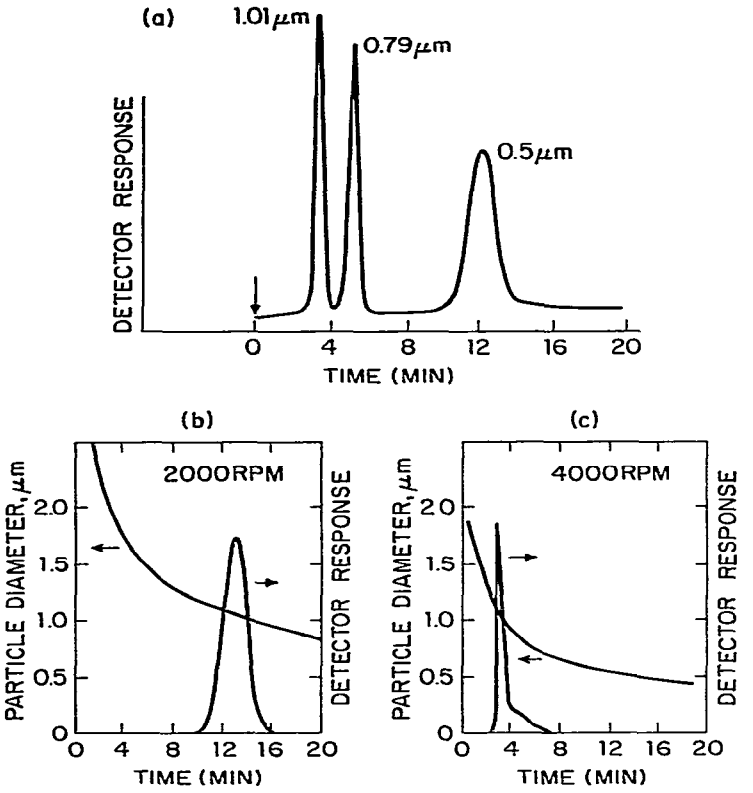

Fig. 5. Disc centrifugation data. 0.5 ml of polystyrene latex mixture dispersion. (a) Separation pattern. Effect of rotor speed on separation: (b) 2000 rpm, (c) 4000 rpm. (Taken from ref. 13.)

SFFF. From the retention characteristics shown in Fig. 5b, the dynamic range of disc centrifugation can be estimated as only about 2, regardless of the field strength used for the separation. As a matter of fact, as shown in Fig. 5c, if the field is too high, the separation range is significantly shifted so that resolution now is seriously degraded. While programming the field strength during analysis would increase the dynamic range of disc centrifugation, such facilities are not currently available in commercial instruments.

Clearly, disc centrifugation exhibits excellent specific resolution, discrimination capacity, and peak capacity, but its dynamic range is relatively small — roughly comparable to CF-SFFF. This limited dynamic range places severe restrictions on the utility of disc centrifugation as a general method for particle size analysis. Also, the high level of operator skill needed with this technique curtails its application.

Disc centrifugation can be used to measure larger particle sizes, up to about 5 μm, compared to about 1 μm for SFFF. Therefore, these methods are somewhat complementary in providing useful data on particles. Because SFFF separates particles according to effective mass while disc centrifugation separates according to sedimentation velocity or particle cross-sectional area, some information regarding particles shape also can be deduced by using both techniques. The Sedigraph is

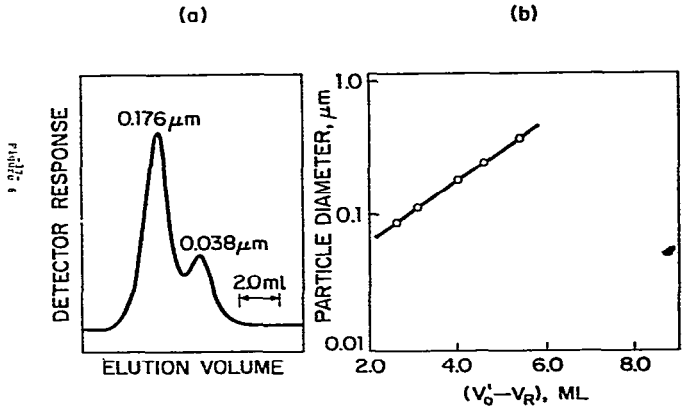

Fig. 6. Column hydrodynamic chromatographic data. (a) Chromatogram; (b) calibration. Polystyrene latices; columns, three 110 × 0.9 cm of non-porous styrene-divinylbenzene beads, 20 μm; mobile phase, 1.29 m*M* sodium dihexylsulfosuccinate; detector, UV. (Taken from ref. 14.)

another sedimentation velocity technique for particle size analysis, but is generally applicable only to larger particles.

Packed-column hydrodynamic chromatography (Col-HDC)

The typical HDC chromatogram shown in Fig. 6a (from ref. 14) exhibits a σ value of 0.68 ml and a D_2 value of 2.11 ml^{-1}. The calibration curve in Fig. 6b indicates a dynamic range of about 5 for Col-HDC. The tabulated data in Table II show that Col-HDC has a much poorer specific resolution, discrimination capacity, and peak capacity than any of the previously discussed methods, but a fairly wide dynamic range is available.

Capillary hydrodynamic chromatography (Cap-HDC)

From the Cap-HDC chromatogram shown in Fig. 7a (from ref. 15), a peak σ value of 4.95 ml, a D_2 value of 0.55 ml^{-1} and a (σD_2) value of 2.7 is calculated. The calibration curve in Fig. 7b shows an effective separation range of 1 μm to about 50 μm, indicating a dynamic range of 50. Based on these values, the performance of Cap-

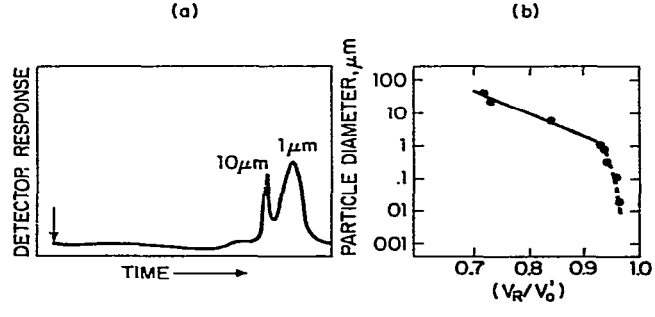

Fig. 7. Capillary hydrodynamic chromatographic data. (a) Chromatogram; (b) calibration. Polystyrene latices; column, 200 ft. × 0.015 in. I.D.; mobile phase, methanol. (Taken from ref. 15.)

HDC was calculated and listed in Table II. This method shows poorer resolution and discrimination capacity than Col-HDC, but a significantly wider dynamic range. However, Cap-HDC clearly is much inferior to all of the other separation techniques in terms of specific resolution, discrimination capacity and peak capacity.

The dynamic range of 50 for Cap-HDC as shown in Fig. 7b seems to be unrealistically large. This reported dynamic range is based on a peak retention volume which elutes 30% earlier than that of the marker peak, that is, $V_R/V_0' = 0.7$ as indicated in Fig. 7b. However, it has been predicted by theory[16] that the earliest possible elution is limited to a volume of only 15% prior to peak elution volume of the total permeating marker peak, V_0'. If indeed the smaller available volume for separation predicted by theory is more typical, Cap-HDC exhibits a smaller dynamic range and an even poorer performance than suggested by data in ref. 15.

Unfortunately, both Col- and Cap-HDC suffer from problems of potential column pluggage and poor solute recovery. In both methods, quantitative particle size distribution calculations are difficult and relatively imprecise because of the necessity for making very large corrections for instrumental peak broadening. In many instances HDC also suffers from undesirable effects as result of changes in solute concentration, flow-rate and mobile phase composition.

Size-exclusion chromatography

The use of SEC for particle size separations has been reported in several publications[17–19]. Published data on polystyrene standards again can be used[19] for comparing the performance of SEC with the various other separation methods. The two chromatograms in Fig. 8a indicate a σD_2 value of about 0.57 for PS standards. The SEC particle size calibration curves shown in Fig. 8b indicate that SEC has a dynamic range DR_{d_p} of about 3 for materials of this type. Calculations with these data provide the resolution performance information summarized in Table II. SEC compares poorly with SFFF and disc centrifugation, but is generally superior to HDC in most areas.

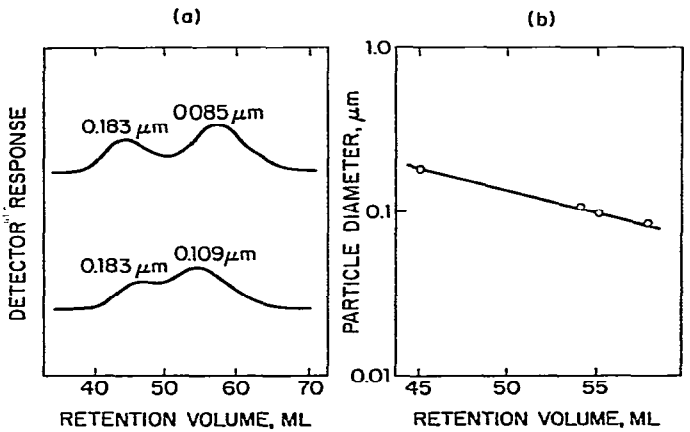

Fig. 8. Size-exclusion chromatographic data. (a) Chromatograms; (b) calibration. Columns: two 2 ft. × 0.35 in., 3000 Å CPG; mobile phase, water with 1 g/l of Aerosol OT and $NaNO_3$; flow-rate, 0.78 ml/min. (Taken from ref. 19.)

Particle size analyses with SEC sometime suffer from column pluggage and poor solute recovery. Also, since the permeation process in SEC requires that solute particles encounter much of an internal porous surface of the column packings, particle size analyses can be complicated by surface adsorption effects[18]. Data handling techniques for SEC also are somewhat complicated, because of significant corrections for peak broadening which must be applied. However, this compares favorably with the extensive peak broadening corrections required for particle size analyses by HDC.

MECHANISTIC VIEW OF RESOLUTION IN SEPARATIONS

The much higher resolution of SFFF documented in Table II compared to SEC and HDC is the result of basic differences in retention mechanisms. SFFF is similar to most liquid chromatography (LC) methods in that all solute particles elute after the unretained solvent peak. In this retentive mode, both LC and SFFF have essentially unlimited retention volumes available for separating sample components. On the other hand, the retention volumes available to SEC and HDC are very limited. Retention in these methods is the result of wall-exclusion effects that cause all solute particles to elute prior to the solvent peak. For SEC, separation is confined within the available pore volume of the column packing, that is, between the interparticle (or total exclusion) volume V_0' and the total permeation volume V_0 as shown in Fig. 9a.

The HDC effect occurs only in the interstitial volume between the column packing particles. Relative to SEC, an even smaller volume is available for separation in HDC, as illustrated in Fig. 9b. The HDC effect is superimposed on SEC retention

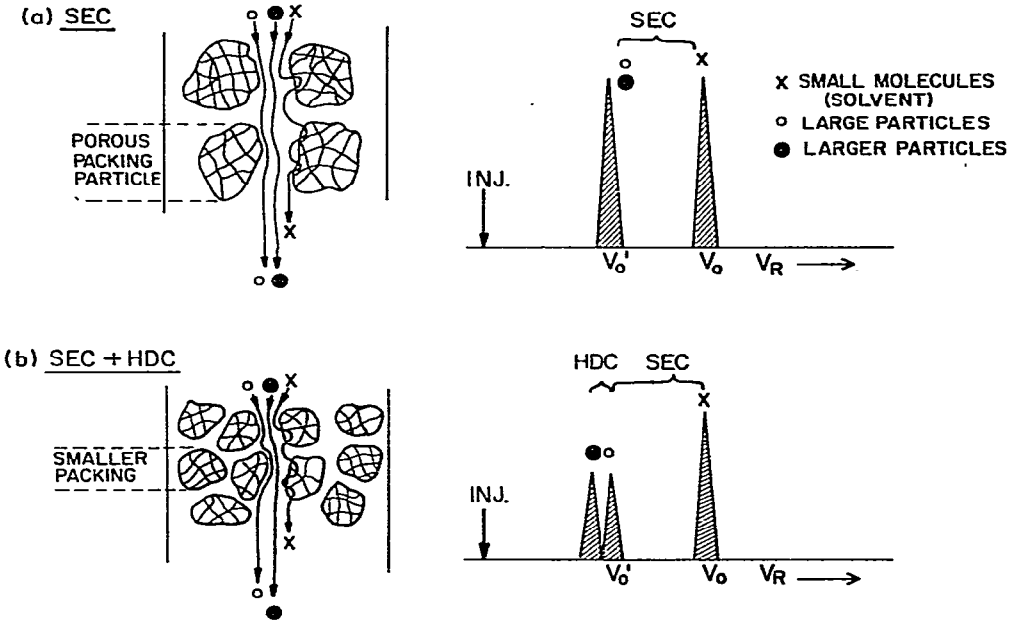

Fig. 9. Comparison of column hydrodynamic and size-exclusion chromatography.

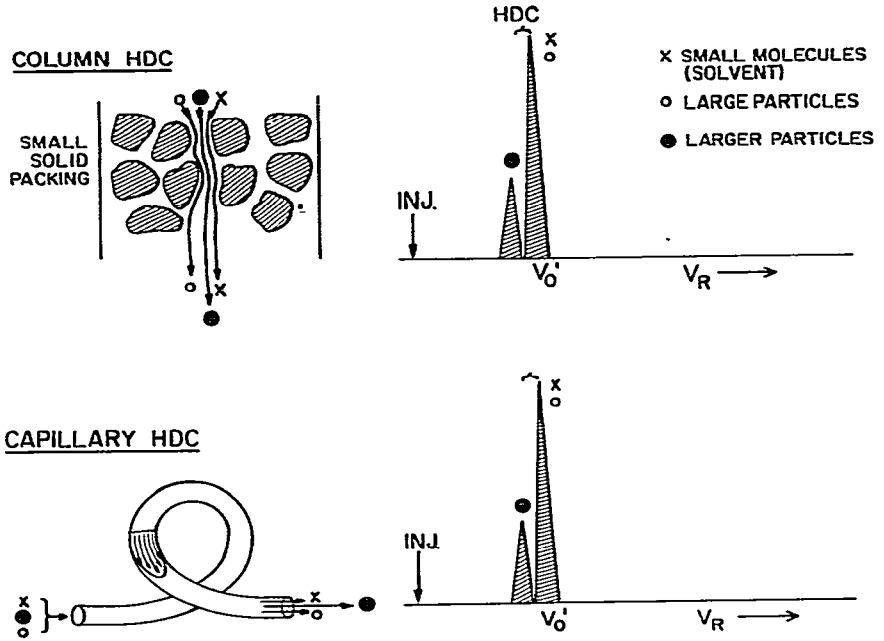

Fig. 10. Comparison of column and capillary hydrodynamic chromatography.

and is only significant when small packing particles are used. In Fig. 10 the elution characteristics of both packed column and capillary HDC are illustrated. A non-porous packing is shown here, but the HDC effect occurs in packed column of both porous and non-porous packing particles. Note that in these methods, just as in SEC, large solute particles elute prior to the mobile phase peak. In both packed-column and capillary HDC the available volume for separation is very small. Only about 15% of the void volume between the column packing particles (*i.e.*, interstitial space) is available for separation. This represents an inherent limitation in the available elution volume range and is directly responsible for the poor resolution of HDC.

On the other hand, SFFF exhibits retention more like LC, with peaks eluting well after the unretained solvent peak. Because of this retentive feature, SFFF has the potential for a very large peak capacity. Fig. 11 illustrates the retention characteristics of SFFF compared to HDC. SFFF is in essence a flow-enhanced equilibrium sedimentation separation. Under an equilibrium sedimentation condition, poorly resolved solute layers are separated by the mobile phase flow which has a laminar (parabolic) velocity profile. With the aid of this flow profile, peaks are highly resolved in SFFF just as in the sedimentation velocity techniques. However, as suggested by the data in Table II, SFFF can have a much higher dynamic range than other methods. In contrast to that found in HDC and SEC, open SFFF channels are expected to be relatively free from pluggage and surface effects.

OTHER PARTICLE SIZE ANALYSIS METHODS

It is also generally feasible to apply the same performance criteria described

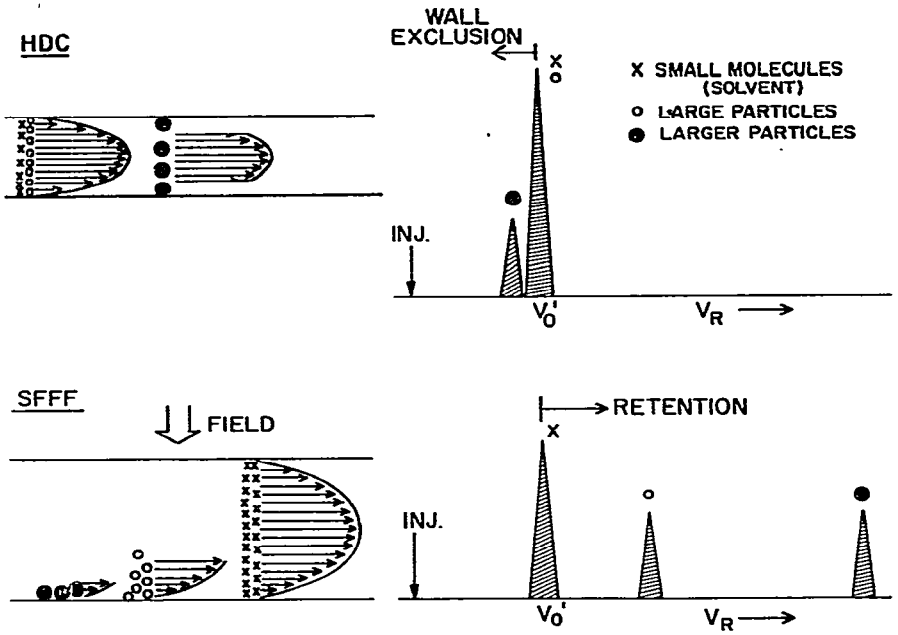

Fig. 11. Comparison of hydrodynamic chromatography and sedimentation field flow fractionation.

above to non-separation particle size analytical methods such as transmission electron microscopy (TEM) and quasi-elastic laser light scattering (LLS) techniques. For example, the Coulter counter is commonly used for determining the size of larger particles, and a typical result is shown in Fig. 12 (taken from ref. 20). In this case, two particle populations with about a seven-fold difference in size are completely resolved. This roughly corresponds to a specific resolution $R_{s,1.2}$ value of 0.04 and a discrimination capacity X_{d_p} value of 5.5, indicating the relatively poor resolution capability of this method. It should be noted that this calculation could be somewhat in error since it

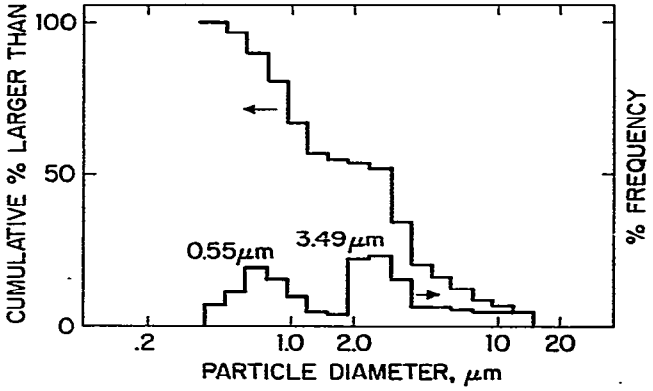

Fig. 12. Coulter counter particle size data. Polystyrene latex standards. (Taken from ref. 20.)

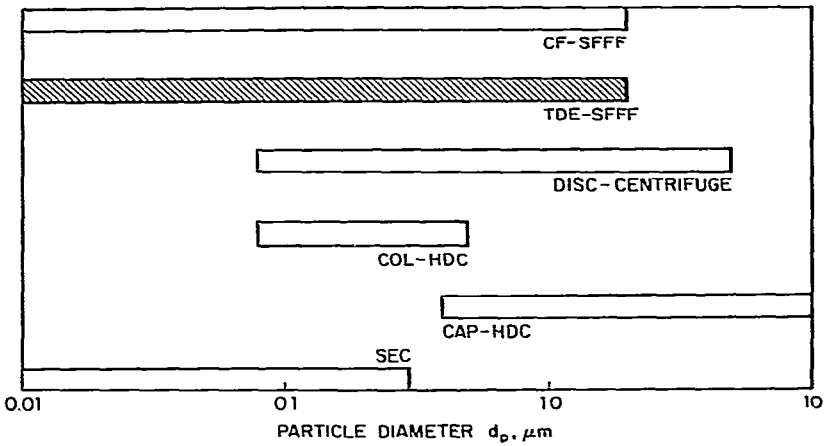

Fig. 13. Accessible particle size range of separation methods.

assumes that the latex particles of each population measured are monodispersed. Nevertheless, it is clear that the resolution of this method is inferior to the other particle size analytical methods listed in Table II. This result suggests that the performance criteria described in this paper can be used for particle size distribution methods other than those based on separations.

Quasi-elastic LLS has been used as a method for rapidly measuring the average size of particles[21]. A computer curve-fitting approach to prepare particle size distribution histograms also has been proposed in an attempt to extract particle size distribution information from the frequency distribution data obtained from light scattering[21]. Unfortunately, the specific resolution of this method is poor. However with the latest techniques, a bimodal distribution has been resolved with a rather poor specific resolution $R_{s,1.2}$ value of 0.3. Also, in this method problems of non-unique solutions sometime occur in the attempt to extract true particle size distributions. The LLS method also suffers from the effect of solute particle concentration dependence and the effect of the angular dependence of the scattered light on particle sizes.

CONCLUSIONS

As illustrated by typical literature data plotted in Fig. 13, SFFF shows a larger total separation range than any of the other separation methods for particle size analysis. However, the more important feature is the range of particle sizes which can be separated in a single experiment, since this feature largely dominates the practical utility of the analytical method. The dynamic range DR_{d_p} of the particle size methods in a single optimum separation is given in Fig. 14a. While Cap-HDC potentially has a wider dynamic range than the other methods, this range is only available for larger particles and at very low resolution.

Fig. 14b has plots of comparative $R_{s,1.2}$ values for the various methods. ($R_{s,1.2}$ distinguishes a 20% difference in particle diameter with a resolution of unity.) SFFF shows approximately equivalent values compared to disc centrifugation, and these methods are highly superior to the others in this regard. For example, TDE-SFFF

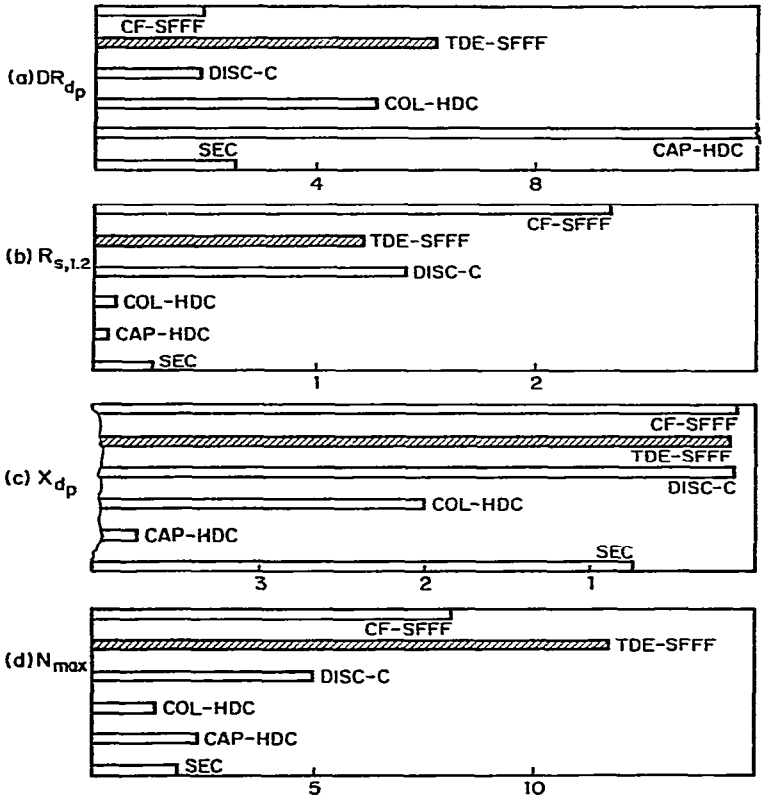

Fig. 14. Comparative resolution performance parameters. (a) Dynamic range, (b) specific resolution, (c) discrimination factor, (d) peak capacity.

shows $R_{s,1.2}$ values which are about 5 times that of SEC and about 11 times that of Col-HDC.

As shown in Fig. 14c, SFFF and disc centrifugation are about equivalent in discrimination capacity X_{d_p}, the lower limit in fractional particle diameter difference that can be discerned by a particular method with a resolution of unity. In terms of X_{d_p} values, SEC and Col-HDC are significantly less effective, followed by Cap-HDC which is very poor in this regard.

TDE-SFFF clearly is shown in Fig. 14d to have a distinct superiority in peak capacity $N_{max.}$, the maximum number of resolved peaks that can fit into the dynamic range of a particular separation. This is an important advantage since it represents the range of particle sizes which can be characterized in a single experiment. Less effective in this regard are CF-SFFF, disc centrifugation, Cap-HDC, SEC and Col-HDC, in that order.

Since numerical values are sometimes difficult to picture, the specific resolutions of the various methods are compared schematically in Fig. 15 for the particle size methods at two specific resolution levels, $R_{s,1.2}$ and $R_{s,3.0}$ (the latter calculated from the exact specific resolution expressions in the Appendix.) At $R_{s,1.2}$, SFFF and disc centrifugation effectively resolves two particles with a 20% difference in diameter; however, no resolution is evident with the other methods. For a three-fold particle size difference at $R_{s,3.0}$, little or no resolution still is characteristic of the HDC

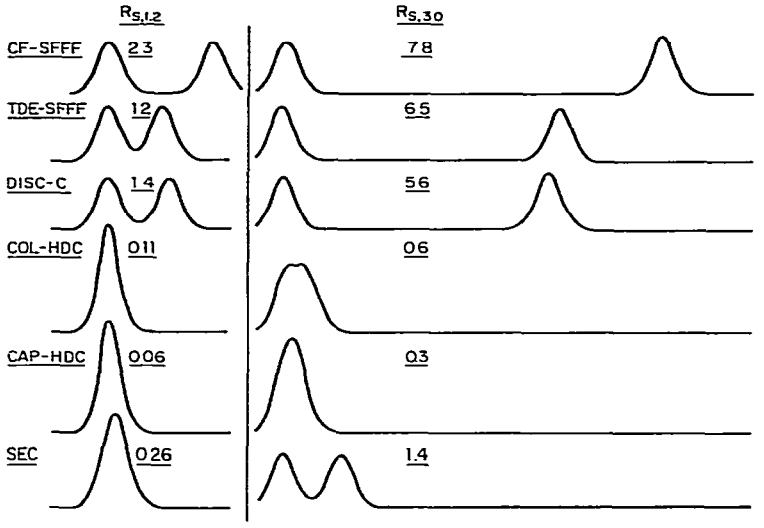

Fig. 15. Comparative resolving power of different separation methods. Left, for $R_{s,1.2}$; right, for $R_{s,3.0}$.

methods and SEC now resolves the two particles. However, TDE-SFFF and disc centrifugation now show enormous resolution for these materials, with CF-SFFF being the most effective.

Clearly, TDE-SFFF is a powerful method for particle size analysis since it possesses a combination of desirable properties, a wide total particle size range, a wide dynamic range for a single experiment, a large specific resolution, a good discrimination factor, and a large peak capacity.

ACKNOWLEDGEMENT

We very much appreciate the critical comments made on this manuscript by D. D. Bly.

APPENDIX

EXACT SPECIFIC RESOLUTION EXPRESSIONS

Case 1 (see Fig. 1 and text)

$$R_s = \frac{V_{R_2} - V_{R_1}}{2(V_{R_2} + V_{R_1})\sqrt{N}} \cong \frac{aM_2^b - aM_1^b}{2\,(aM_2^b + aM_1^b)/\sqrt{N}}$$

$$= \frac{\sqrt{N}}{2}\left(\frac{M_2^b - M_1^b}{M_2^b + M_1^b}\right) = \frac{\sqrt{N}}{2}\left[\frac{(M_2/M_1)^b - 1}{(M_2/M_1)^b + 1}\right]$$

For particle mass:

$$R_{s,X} = \frac{\sqrt{N}}{2}\left[\frac{X^{|b|} - 1}{X^{|b|} + 1}\right] \qquad \text{(Ia)}$$

For particle diameter:

$$R_{s,x} = \frac{N}{2} \frac{X^{3|b|} - 1}{X^{3|b|} + 1} \tag{Ib}$$

where X is ratio of particle masses or particle diameters for a pair of reference particle species. For example, an $R_{s,3}$ value for particle diameter separation describes the expected resolution for a pair of particles with a three-fold difference in particle diameter.

Case II (see Fig. 2 and text)
For particle mass:

$$R_{s,x} = \frac{|c|\ \Delta \ln M}{4\sigma} = \frac{|c|\ \ln X}{4\sigma} \tag{IIa}$$

For particle diameter:

$$R_{s,x} = \frac{3|c| \cdot \ln X}{4\sigma} \tag{IIb}$$

REFERENCES

1 F. J. F. Yang, M. N. Myers and J. C. Giddings, *Anal. Chem.*, 46 (1974) 1924.
2 J. C. Giddings, L. K. Smith and M. N. Myers, *Anal. Chem.*, 48 (1976) 1587.
3 F. J. F. Yang, M. N. Myers and J. C. Giddings, *J. Colloid Interface Sci.*, 60 (1977) 574.
4 J. J. Kirkland, W. W. Yau, W. A. Doerner and J. W. Grant, *Anal. Chem.*, 52 (1980) 1944.
5 W. W. Yau and J. J. Kirkland, *Sep. Sci. Technol.*, 1981, in press.
6 J. J. Kirkland, S. W. Rementer and W. W. Yau, *Anal. Chem.*, 1981, in press.
7 J. J. Kirkland, W. W. Yau and F. C. Szoka, *Science*, 1981, in press.
8 W. W. Yau, J. J. Kirkland and D. D. Bly, *Modern Size-Exclusion Chromatography*, Wiley, New York, 1979, Ch. 4.
9 W. W. Yau, J. J. Kirkland, D. D. Bly and H. J. Stoklosa, *J. Chromatogr.*, 125 (1976) 219.
10 E. Pfannkock, K. C. Lu, F. E. Regnier and H. G. Barth, *J. Chromatogr. Sci.*, 18 (1980) 430.
11 J. C. Giddings, *Anal. Chem.*, 39 (1967) 1027.
12 W. W. Yau, J. J. Kirkland and D. D. Bly, *Modern Size-Exclusion Chromatography*, Wiley, New York, 1979, Ch. 9.
13 T. Provder and R. M. Holsworth, *Preprints, 172nd American Chemical Society Meeting, San Francisco*, 32 (2) (1976) 150.
14 A. J. McHugh, D. J. Nagy and C. A. Silebi, in T. Provder (Editor), *Size-Exclusion Chromatography (GPC), A.C.S. Sym. Ser.* 138, Washington, DC, 1980.
15 R. J. Noel, K. M. Gooding, F. E. Regnier, D. M. Ball, C. Orr and M. E. Mullins, *J. Chromatogr.*, 166 (1978) 373.
16 C. Orr, Jr., in M. J. Groves (Editor), *Particle Size Analysis*, Heyden, London, 1978, p. 92.
17 H. Coll, G. R. Fague and K. A. Robillard, *Exclusion Chromatography of Colloidal Dispersions*, unpublished results, 1975.
18 J. J. Kirkland, *J. Chromatogr.*, 185 (1979) 273.
19 A. Husain, A. E. Hamielec and J. Vlachopoulos, in T. Provder (Editor), *Size-Exclusion Chromatography (GPC), A.C.S. Symposium Series* 138, Washington, DC, 1980.
20 E. A. Collins, J. A. Davidson and C. A. Daniels, *J. Paint Technol.*, 47 (1975) 35.