ELECTRICAL SIZING OF PARTICLES IN SUSPENSIONS

II. Experiments With Rigid Spheres

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ABSTRACT Experimental verification is provided for the theoretical expressions (see preceding article, I. THEORY) describing the electrical processes that take place during the passage of an aqueous suspension of rigid, nonconducting spheres (ragweed pollen) through an orifice across which there exists an electrical field, for a large range of orifice dimensions; the instrumentation developed is considered in some detail. The effective length of an orifice as deduced from conductivity measurements is shown to be essentially the same as that predicted theoretically. Absolute volume distributions are presented of a suspension of polystyrene latex spheres as determined electrically (mean 11.17 μ^3, c. v. 4.2%) and with an electron microscope (mean 11.01 μ^3, c. v. 4.1%). Conflicting experimental results reported in the literature are discussed.

INTRODUCTION

In the physical sciences, there are several techniques available for determining particle size distributions (1); the sizing of living cells, however, is much more difficult, and in practice only two methods have been used. Optical microscopy is of course the most direct approach, but it suffers from several limitations (2) and is very tedious. The inherent variability of biological populations requires larger samples than is generally practicable with this method. The second method is electrical in nature and is capable of high sampling rates and, at least in theory, of very high resolution. This is the electronic cell counter developed by Coulter (3), the principle of which is based on the change in electrical resistance that occurs when a particle suspended in a conducting medium is pumped through a narrow orifice across which there exists an electric field.

A disturbing feature of electrical sizing is the contradictory results of particle (4–6) and cell (6–9) size distributions obtained under different experimental conditions (electrical fields, orifice dimensions, flow rates); owing to the lack of specifications furnished with the commercial instrument, it is not possible to decide whether such discrepancies are due to improper theoretical considerations or to experimental limitations. These problems can be resolved only by formulating a theory adequate to cover the range of experimental conditions encountered in practice and by designing and building a measuring system accurate enough and flexible enough to permit critical testing of the theory. In particular one must be able to compare the change in resistance within the orifice during the passage of a particle as predicted by the theory with that determined by measuring the signal generated at the output end of the device—calibrating particles should not be necessary.

The first serious attempt in this direction, as regards both theory (10) and experiment (2), was made by Gregg and coworkers. These researchers designed their own electronic counter using the Coulter orifice and compared their results with microscopic measurements on intact, undistorted, living cells in suspension as well as with two types of pollen. In all cases the mean volumes determined electrically were substantially smaller than those measured optically. The authors attribute this to the inadequacy of the optical method, but some of the discrepancy is probably due to their choice of orifice dimensions—a question not dealt with by their simplified theory.

A more detailed theoretical treatment has been presented previously (11); in the present paper we first consider the experimental aspects of the problem and then test the results obtained against the theory for the case of rigid spheres.

INSTRUMENTATION

The measuring system developed[1] to study the processes involved during the passage of a particle through an orifice is illustrated in block diagram form in Fig. 1. The transducer is similar to that used in the Coulter counter except that the orifices could be ordered[2] with any desired length and diameter. Stability and low noise were achieved by using completely solid-state circuitry adapted from standard nuclear instrumentation. In order to prevent amplifier distortion even with low-conductivity electrolytes and large parasitic capacitances, a constant-current power supply was used in conjunction with a current amplifier in the preamplifier stage. The supply provides calibrated currents of either polarity from 20 μa to 20 ma, in 11 steps, together with an uncalibrated fine control between steps. A current change of 1 μa in the orifice results in a voltage change at the preamplifier output of 1 v.

The amplifier is a standard bipolar linear amplifier with a nine-step calibrated gain factor of 4 to 1000 and an uncalibrated fine control for interpolation. Its integration time is selectable from 0.2 μsec to 20 μsec in six calibrated steps. The lower values are for use with fast pulses (short orifices) while the higher values provide the possibility of improving the signal-to-noise ratio when working with longer orifices.

The gate generator and peak detector circuits contain three controls. The threshold control selects the minimum signal required to activate the peak detector (0.2–10 v), and is useful in discriminating against noise and in particle counting. The other two controls determine

[1] Yissum Research Development Company, The Hebrew Univvrsity, Jerusalem

[2] Swiss Jewel Co. S.A., Locarno

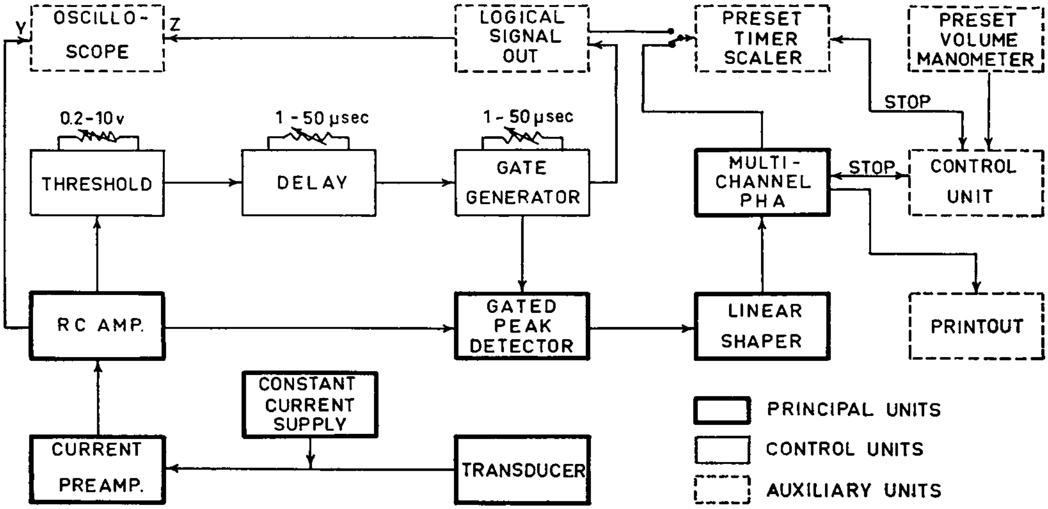

FIGURE 1 Block diagram of experimental apparatus for electrical sizing of particles, and associated instrumentation.

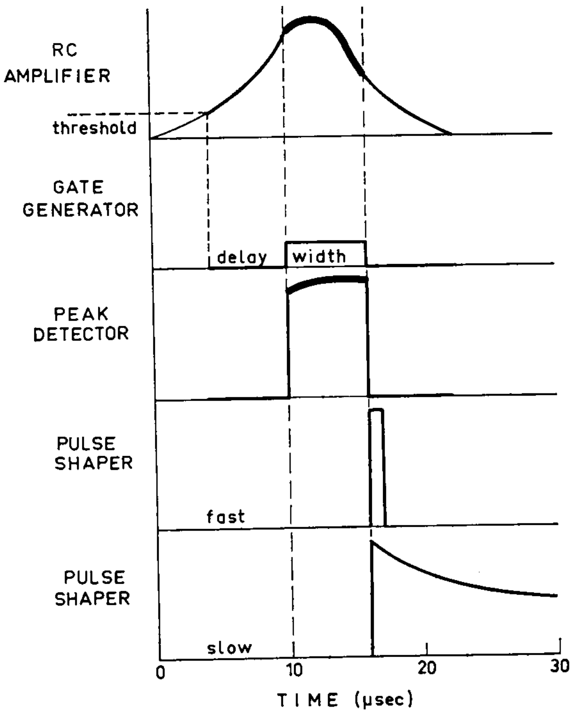

FIGURE 2 Schematic diagram of the shaping of a pulse, illustrating operation of the gate generator and peak detector circuits.

the delay (1–50 μsec) from the time the signal crosses the threshold until the peak detector is activated and the length of time (1–50 μsec) that it remains in the active state. These controls are essential when studying the signals developed at different points along the length of the orifice. The amplified signal can be viewed on an oscilloscope, and a modulation signal is provided which enhances that portion of the trace that coincides with the period of activation of the peak detector.

The measuring system is terminated by a 100-channel multichannel pulse-height analyzer with paper tape and hard copy outputs. In order to ensure proper response of the analyzer, it is fed pulses of standard shape and width provided by a pulse-shaping network at the output of the peak detector; the amplitude of each pulse is equal to the maximum height reached by the amplified signal within the time interval selected by the gate generator (Fig. 2). A scalar is attached to the analyzer and control circuits permit measuring at preset count, preset real time, preset live time, and preset volume of suspension transported through the orifice.

RESULTS

A calibrated variable pulse generator with independently adjustable rise and decay times was used as a pulsed current source to simulate the passage of particles through the orifice, and the linearity of the measuring system was verified for both polarities over the entire dynamic range of the amplifiers; the design of the preamplifier as a current amplifier resulted in the amplification being independent of orifice resistance from 1 kΩ to 1 MΩ and of parasitic capacitance up to 0.1 μf.

Synthetic rubies with cylindrical holes were used as orifices. Their diameters at both ends were measured microscopically to within $\frac{1}{2}$ μ (such high accuracy can be obtained by filling the holes with a colored solution—a procedure that improves considerably the sharpness of the hole's boundaries) and their lengths mechanically to within 2 μ before mounting[3] over holes in pyrex tubes. The effective volume V of an orifice is given by $\pi R^2 l$, where l is the effective length:

$$l = L + 1.64R + \epsilon. \tag{1}$$

Here L is the geometrical length of the orifice and R its radius. The second term expresses the contribution to l of edge effects (12) whereas the third term, represented by ϵ, contains the contribution of the regions between the orifice and the electrodes. In the configurations usually used, the contribution of the outer region (from which the suspension is pumped) is negligible while that of the inner region is at most a few per cent of L. Thus an estimate of ϵ is sufficient, and this can be had by approximating the inner region by several cylinders in series each of which contributes to ϵ by an amount equal to its length divided by the ratio of its cross-sectional area to that of the orifice.

A simple, though nongeometrical method of determining l is by measuring the

[3] Armstrong A271 epoxy cement, Armstrong Products Co. Inc., Warsaw, Ind.

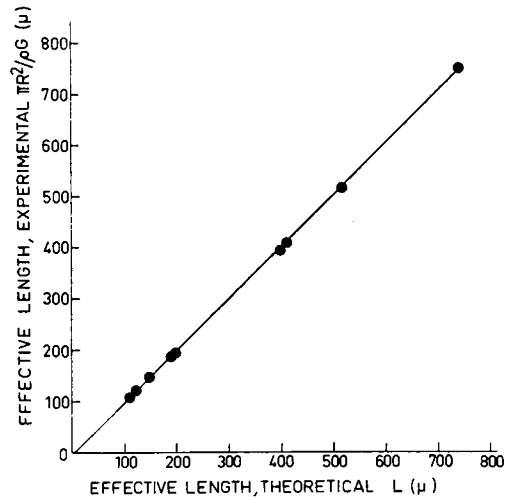

FIGURE 3 Plot of $\pi R^2/\rho G$, the effective orifice length as determined from conductivity measurements, as a fuuction of the calculated value l.

conductance G between the electrodes using an electrolyte of resistivity ρ:

$$l = \frac{\pi R^2}{\rho G}. \tag{2}$$

Fig. 3 shows a plot of $\pi R^2/\rho G$ against corresponding values of l as determined from equation 1 for nine different orifices. (Measurements were made at 10 kHz using 0.1 N KCl as the electrolyte.) The excellent fit with the theoretical curve lends confidence to the assumption that equation 2 provides an equivalent means of defining the effective length, and since experimental determinations based on equation 2 are capable of furnishing data that are more precise than those based on equation 1, in the discussion that follows orifice volumes are calculated from values of l obtained from equation 2.

In the present paper we avoid the effects of deformation and orientation and restrict ourselves to rigid spheres. The theory predicts (11) that the relative current change $\Delta I/I$ generated during the passage of such particles through an orifice of effective volume V be

$$\frac{\Delta I}{I} = -\gamma \frac{v}{cV - v} \tag{3}$$

provided that ΔI is measured when the particle is far from the mouth of the orifice, where the electric field is uniform, and provided that

$$3(a/R')^{10} \ll 1, \tag{4}$$

where a is the radius of the particle and R' is the radius of the largest concentric

TABLE I

EXPERIMENTAL PARAMETERS IN DETERMINATION OF VOLUME OF RAGWEED POLLEN PARTICLES IN VARIOUS ORIFICES

Orifice dimensions (nominal)		Effective volume	Orifice current	System amplification	Distribution mean	Mean size
R	L	V	I	–	–	$\gamma v/(c - v/V)$
μ	μ	$\mu^3 \times 10^5$	μa	*channels/μa*	*channel*	μ^3
50	80	2.702	107	22.84	42.17	4662
			124		49.56	4728
			124		50.28	4797
50	100	3.451	143	22.84	44.32	4683
			143		46.82	4947
			143		46.64	4922
50	150	4.307	172	22.84	42.63	4674
			173		44.06	4803
100	200	23.95	689	22.84	29.87	4546
			687		30.20	4610
			1040		44.80	4517
100	300	31.49	862	22.84	28.26	4520
			1335		44.75	4622
200	300	175.9	5000	22.84	32.26	4969
			1160	89.28	27.17	4615
			1930		46.01	4697
			1900		45.31	4698
200	400	261.8	2620	89.28	44.65	4997
			1780		29.21	4812
			2580		43.64	4960

sphere in which the electric field is uniform in the absence of the particle. Here v is the volume of the particle and γ its shape factor ($= 1.5$ for spheres). The parameter c is a function of ω, the ratio of the resistivities of the suspended and suspending medium:

$$c = 1 + \frac{\gamma}{\omega - 1}; \tag{5}$$

for nonconducting particles ($\omega \gg 1$), c approaches unity.

It was desired to test equation 3 for many different orifices over a large range of V, and ragweed pollen particles[4] were selected for this purpose because they are rigid spheres of reasonably uniform volume (c. v. $< 15\%$) that are big enough to provide adequate sensitivity with our larger orifices while still satisfying equation 4 for orifices 100 times smaller. The ragweed was freshly mixed with phosphate-buffered saline and briefly sonicated (40/sec) in order to obtain a monodisperse suspension of intact particles. About 20,000 particles were measured in each experi-

[4] Particle Information Service, 600 South Springer Road, Los Altos, Calif.

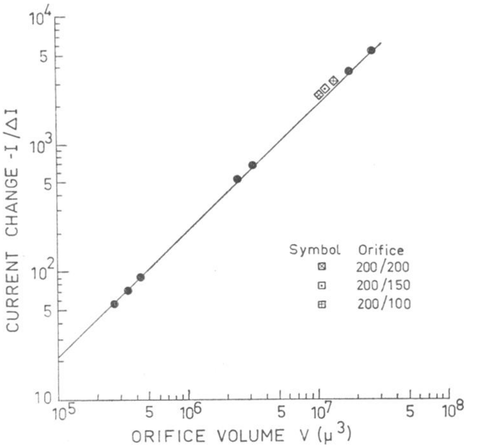

FIGURE 4 Plot of $-I/\Delta I$, the reciprocal of average signal generated by particles as they pass through an orifice, as a function of effective orifice volume V for a suspension of monodisperse ragweed pollen. Numbers in legend are in microns and refer to nominal dimensions (diameter/length) of orifices. Circles: inequality of equation 4 completely satisfied. Squares: inequality of equation 4 not completely satisfied. Solid curve: best straight line through circles. Log–log scale has been chosen for convenience of presentation.

ment, the concentrations being such as to reduce coincidence (the simultaneous presence within the orifice of more than one particle) to less than 1 %.

The experimental details and results of several typical runs are given in Table I. Mean values are reported rather than modes although the size distributions are unimodal and quite symmetrical and so there is little difference between the two. The mean *electrical size* $\gamma v/(c - v/V)$ given in the last column (11) has been calculated from equation 3; it appears to be independent of orifice current over the range studied. (The complete current range was not covered because of certain disturbances of unknown origin that began to appear in the output signal of the amplifier at very high currents.) The average of the means is 4739 μ^3, with a coefficient of variation of less than 3 %. Most of this rather small spread can be accounted for by the uncertainties in the determination of V from R and G; results obtained on the same orifice are even more homogeneous.

The results in Table I have been averaged for each orifice and a linear least squares fit calculated for $-I/\Delta I$ as a function of V. The fit obtained is excellent (the correlation coefficient = 0.9998), thereby confirming the predicted dependence on orifice volume of the signal generated by rigid spheres. The slope of the theoretical line, which by inverting equation 3 is seen to be $c/\gamma\bar{v}$, is equal to $(204.7 \pm 4.8) \times 10^{-6}\ \mu^{-3}$ while the intercept $(-1/\gamma)$ is equal to 22 ± 57. The ranges quoted represent the 95 % confidence limits and show that the slope can be determined with high accuracy whereas the intercept cannot be distinguished from zero because $\gamma\bar{v} \ll cV$ for all values of V and indeed must be so in order to satisfy both equation 4 and the requirement of uniform electric field. Thus one cannot determine $\bar{v}$ and γ separately by this method but only their product (by using nonconducting particles and setting c to unity) and another, independent measurement is necessary. Such a measurement and its results are described below. (For purposes of comparison, the experimental results (circles) and the regression line are shown in Fig.

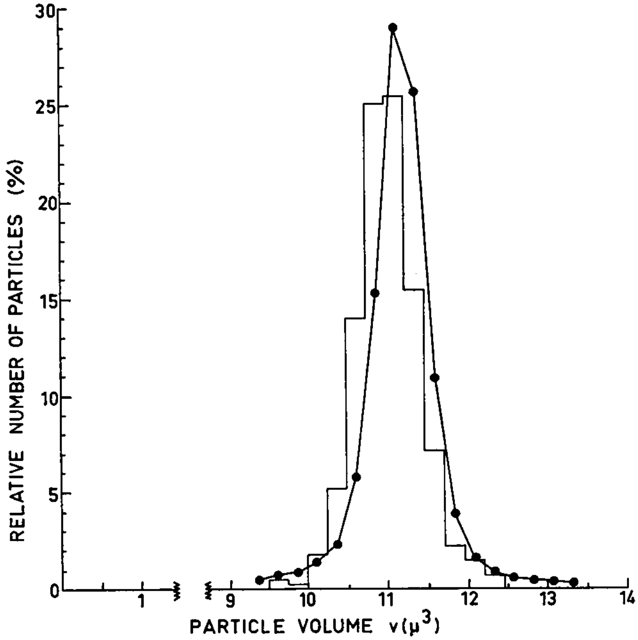

FIGURE 5 Volume distribution of polystyrene latex particles as determined electrically (broken line) and with electron microscope (histogram).

4 together with three points (squares) obtained from measurements with orifices in which the inequality of equation 4 is not completely satisfied. Owing to the large range of values covered, the data are presented in log–log form, such a transformation being made possible by the fact that the intercept of the theoretical fit is indistinguishable from zero.)

The most direct means of measuring particle volume is microscopically. Because of the tediousness of the method, however, the size of the sample is limited by practical considerations, and sufficient accuracy can be attained only with very uniform particles. In addition the surface of the particle must be optically well defined and regular. This latter condition is not met by the ragweed pollen; in fact the only particle we were able to find that does meet the requirements is polystyrene latex particles[5] with a very narrow volume distribution (c. v. $< 5\%$), and these were employed instead despite their small size (diameter $< 3\ \mu$), which necessitated using the electron microscope.

The latex particles were suspended in distilled water, deposited on a carbon-coated copper grid, and photographed (electron magnification $\times$ 3000) on glass plates. The images were then projected onto graph paper (1 mm grid) and the meas-

[5] Dow Physical Research Laboratory, Dow Chemical International Limited S.A., Midland, Mich.

urements made, the final magnifications ($\times$ 30,000) having been determined for each plate by calibration against the photograph of a grating replica[6] taken on that plate. Three different diameters were measured for every particle, the accuracy of each determination being $\pm\frac{1}{2}$ mm.

The volume distribution of a sample of 1250 particles was calculated and found to consist of two populations: the first containing about 90% of the particles and having a mean of 11.01 μ^3, the second being widely scattered with a mean around 25 μ^3.

Latex particles from the same batch were then taken and suspended in an electrolyte (NaCl). After sonication, the suspension was introduced into the apparatus described in the previous section and the size distribution determined under the following conditions: orifice diameter 30 μ, orifice length 80 μ (nominal dimensions), orifice current 1.32 ma, amplification 89.3 channels/μa, sample size 15,000 particles.

The value obtained for $\gamma\bar{v}/c$ under these conditions is 16.75 μ^3, and by substituting 11.01 μ^3 for $\bar{v}$ from the electron microscope measurements and putting c = 1, one finds that γ equals 1.52. This is very close to the theoretical value of 1.5 and suggests that the difference between the two is due to experimental error rather than to any inadequacy in the theory.

If we now assume that the shape factor for spheres is 1.5 and substitute this for γ, we obtain for $\bar{v}$ a value of 11.17 μ^3. Fig. 5 is a plot of the volume distribution of polystyrene latex particles as determined electrically (broken line) and with the electron microscope (histogram). Not only are the means nearly equal (11.17 μ^3 compared to 11.01 μ^3), but so are the coefficients of variation (4.2% compared to 4.1%).

Thus the instrument described in this paper is capable of measuring accurately not only particle volume but also the particle volume distribution.

DISCUSSION

The electrical sizing of particles does not provide the means for distinguishing between the shape factor of the particles γ and their mean volume $\bar{v}$, only the product $\gamma\bar{v}$ is obtained. In order to separate the two parameters, the mean volume of a homogeneous population of polystyrene latex spheres was determined from electron microscopic measurements; the value subsequently calculated for γ differed by less than 2% from the theoretical value of 1.5.

We can now consider the theoretical value of γ for the case of spherical particles as having been established experimentally. Replacing γ by 1.5 in the value of $\gamma\bar{v}$ determined from the linear fit shown in Fig. 4 (by setting c to unity, as for all nonconducting particles), we obtain a mean volume for ragweed pollen particles of

[6] Ernest F. Fullam Inc., P.O. Box 444, Schenectady, N. Y.

3257 μ^3. This value of $\bar{v}$ is much smaller than the generally accepted optical value, although there seems to be quite a variation in the latter (13). The difference between the volume as determined optically and as determined electrically is probably due to the irregularities on the surface of the ragweed pollen, which tend to exaggerate the apparent diameter as seen under the microscope (2). Any porous regions present in the particle will also contribute to the decrease in electrical volume, as is the case with certain bacteria (14).

An even larger discrepancy between the two volumes has been reported by Gregg and coworkers (2). In addition to the inherent differences described above, in this case there was probably also an underestimation of the actual electrical volume, and for two reasons. First of all, the length of the orifice used (70 μ) was significantly less than its diameter (100 μ). As a result, nowhere within the orifice was the electric field sufficiently homogeneous (11) to satisfy their assumptions (10) or the requirements of equation 4, the relationship between the signal developed by the passage of a particle and the volume of the particle thus remaining undefined. Experimentally one can show (see Fig. 4) that values of $\bar{v}$ obtained with such short orifices are too small by about 15%.

The second reason for underestimating the electrical volumes is instrumental. When the flow rates quoted are used with a 70-μ-long orifice, most of the signals are bell-shaped and reach their peak in only 7 or 8 μsec as the particles pass the middle of the orifice. Under such conditions, some signal reduction can be expected from an amplifier with a risetime of 5 μsec. (This problem is more serious in instruments using voltage amplifiers because there the effective risetimes may be even larger, at least with high resistance orifices, due to the presence of parasitic capacitance at the input of the amplifiers.)

Other investigators (4–6) have attempted to determine the reliability of electronic cell sizing by using polystyrene latex spheres and comparing the size distributions obtained electronically with that from electron microscopic measurements or with data furnished by the manufacturer; in either case comparisons were based on the coefficient of variation of the distribution and on its symmetry.

Using these criteria, Van Dilla (6) found that very long orifices (30 μ diameter, 225 μ length) give true results while shorter ones (30 μ by 59 μ) do not; Edwards and Wilke (5) employ the short orifices available commercially and also report discrepancies, while Harvey and Marr (4) describe an apparatus that gives true results even with such short orifices.

When one considers the completely featureless pattern of the volume distribution of polystyrene latex spheres (Fig. 5) and the fact that any increase in the coefficient of variation can always be explained as being due to electronic noise (6) or to theoretical idealizations (5), it is difficult to see how any valid conclusions can be drawn concerning the correctness of a particular experimental configuration based on these criteria. The extension of any such conclusions to other types of particles is even less justified.

The present paper has shown that a detailed theoretical analysis of the processes taking place during the passage of a particle through an orifice across which there exists an electric field is capable of defining the experimental conditions under which, with appropriate instrumentation, accurate information can be obtained on the volume distribution of rigid, nonconducting spheres. This provides a basis for the investigation of the volume distribution of living cells—research now in progress and to be reported on in a subsequent publication.

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