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Counting and Sizing of Submicron Particles by the Resistive Pulse Technique

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The resistive (Coulter) method of counting and sizing particles in a conducting fluid has been extended to polystyrene spheres 900 Å in diameter, with a present detection limit near 600 Å, through the use of individual submicron pores etched in irradiated plastic sheet. The use of a nonionic surfactant and ultrasonic cleaning effectively relieves the problem of plugging. The particles may be driven through the pore by the electric field, without the use of pressure, to yield the vector sum of the electrophoretic and electro-osmotic velocities. A new theory, yielding an upper limit to the resistive pulse on passage of a sphere, agrees well with data for spheres with diameters $d<0.4D$, where D is the pore diameter, and complements a previous theory that gives a lower limit, valid for $d>0.9D$. We estimate that a detection limit near 250 Å will be attainable with the further development of current techniques.

INTRODUCTION

THE need to count and size individual microscopic particles exists in a wide variety of fields. The Coulter counter (Coulter Electronics, Incorporated, Hialeah, Florida) is a device that performs this function automatically for particles suspended in an electrolyte.[1] (Kubitschek[2] has recently reviewed the theory and use of this apparatus.) In this instrument the particles are caused to flow, by pressure difference, through a small current carrying aperture. The momentary changes of voltage that occur as the particles pass one by one through the aperture are counted and classified according to magnitude. These small pulses arise from the momentary displacement of the electrolyte by the relatively insulating particle and are closely proportional to the volume of each particle. The constant of proportionality depends somewhat on the shape, aspect, and size of the particle.[3]

The present lower particle size limit for the commercial counter is about 0.5 μ in diameter. On a laboratory basis, Kubitschek[4] has detected particles as small as 0.2 μ. We report here the routine detection of 0.09 μ particles. Since the signal is proportional to the cube of the diameter, this represents an order of magnitude increase in sensitivity, and further increases appear feasible. Thus, the way appears to be open, for example, for automatically sizing and counting medium sized viruses, or for analyzing contaminants too small to be resolved individually by light scattering devices.

An important new feature that adds another dimension for characterizing particles is that they may be driven through the aperture electrophoretically, without the use of any pressure difference. The time of passage constitutes the added parameter. In the present paper we merely note the occurrence of this effect and reserve complete description and discussion for a later communication.

The increased sensitivity has been made possible through the development of Nuclepore filter material at our Laboratory.[5] Uniform pores of any diameter from about 0.010 μ on up may be etched through nonconducting materials, such as Lexan polycarbonate plastic sheets, where high energy nuclear particles have left damage tracks. Individual pores of submicron diameter are isolated and used as apertures.

THEORY

The central theoretical problem is that of determining the increase in resistance of a conducting circular cylinder caused by the insertion of an insulating sphere far from the ends. There is no exact solution for this problem. Two approximate solutions have been proposed. We shall review these and introduce a third solution that appears to have a wider range of application than the earlier approximations.

Maxwell,[6] using an exceptionally ingenious argument, obtained an expression for the effective resistivity, ρ_{eff}, of a dilute suspension of insulating spheres in a solution of resistivity ρ. Put in terms of the volume fraction f of the

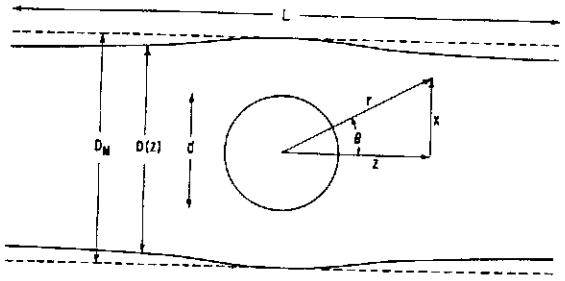

FIG. 1. $D(Z)$ shows the axial cross section of an electric field streamline tube of length L, as distorted by insertion of a nonconducting sphere of diameter d, placed in a conducting fluid with an originally uniform field along the Z axis. D_m represents the cross section of a uniform tube with a diameter equal to that of the distorted tube at its maximum bulge.

spheres in suspension, Maxwell's approximation is

$$\rho_{eff}=\rho(1+3f/2+\cdots). \tag{1}$$

Later, Lord Rayleigh[7] obtained the same expression in an explicit calculation for a dilute cubic lattice of spheres.

If we consider a tube of diameter D and length L filled with a fluid of resistivity ρ, then the resistance for $L\gg D$ approaches

$$R_1=4\rho L/\pi D^2. \tag{2}$$

[For L comparable to D, one should substitute $(L+0.8D)$ for L to make an approximate correction for end effects.[8]] If a sphere of diameter d is introduced, then the volume fraction of this sphere to the total tube volume is

$$f=2d^3/3D^2L. \tag{3}$$

By Eqs. (1)–(3), we obtain for the resistance R_2 of a tube with a small sphere within it

$$R_2=4\rho_{eff}L/\pi D^2$$
$$=(4\rho L/\pi D^2)(1+d^3/D^2L+\cdots). \tag{4}$$

Thus, the increase in resistance, ΔR, is given by

$$\Delta R=R_2-R_1=4\rho d^3/\pi D^4 \tag{5}$$

in the limit that the diameter of the sphere is much smaller than the diameter of the tube or pore. This expression shows that the resistive pulse is proportional to the cube of the diameter and inversely proportional to the fourth power of the pore diameter. Thus this effect can be used either to size particles or to measure pore diameters. We shall give examples of both uses later. The expression for the resistive pulse is seen to be independent of pore length for a long pore, as is obvious on physical grounds.

While Eq. (5) is correct for a sphere small in comparison to the tube diameter, we need an expression that is valid over a broader range. A complete solution of this problem requires a solution of Laplace's equation for the potential subject to insulating boundary conditions at both the sphere and cylinder boundaries. Barring this solution, one must use approximations. One of the most useful approximations for the resistance of a tube of varying cross section is

$$R=\rho\int\frac{dz}{A(z)}, \tag{6}$$

where $A(z)$ is the cross sectional area perpendicular to a length coordinate z. As Maxwell notes,[9] this gives a lower bound to the resistance owing to the fact that while the total current is conserved, the distribution is assumed uniform across a cross section. Any nonuniformity of current density will give a larger resistance. As to its applicability, in Maxwell's words, "This method in the case of wires whose section varies slowly with length gives a result very near the truth." Kubitschek[4] and, later, Gregg and Steidley[3] have used this approximation in deriving R_2, the resistance of the cylinder containing a sphere. The elements of area in Eq. (6) are the annular rings of solution between the surfaces of the sphere and the cylinder. The complete solution by Gregg and Steidley gives, in our notation,

$$\Delta R=\frac{4\rho}{\pi D}\left[\frac{\sin^{-1}(d/D)}{[1-(d/D)^2]^{\frac{1}{2}}}-\frac{d}{D}\right], \tag{7}$$

or, through expansion in powers of the sphere's diameter,

$$\Delta R=\frac{8\rho d^3}{3\pi D^4}\left[1+\frac{4}{5}\left(\frac{d}{D}\right)^2+\frac{24}{35}\left(\frac{d}{D}\right)^4+\frac{169}{280}\left(\frac{d}{D}\right)^6+\cdots\right]. \tag{8}$$

[In this last expression, the terms in $(d/D)^4$ and $(d/D)^6$ correct slight numerical errors in the expressions of Kubitschek and Gregg and Steidley.] The limiting behavior gives a resistance less than the Maxwellian value [Eq. (5)] by a factor of two thirds. Thus it is, as expected, an underestimate. Recognizing this fact, Gregg and Steidley proposed multiplying their solution by three halves. They obtained some justification for this procedure in a moderate agreement between the expression so amended and model experiments. As will be seen below, we have some reservations concerning this approximation. On the other hand, when the diameter of the sphere is very nearly equal to that of the pore, the extra resistance is localized in the small annular region between the sphere surface and cylinder wall. This section changes slowly with distance in Maxwell's sense, and, hence, Gregg and Steidley's uncorrected solution should be asymptotically valid for large spheres. We shall present experimental evidence for this point of view later.

To attack the problem in another way, we can consider a sphere in a uniform field. A solution of Laplace's equation that permits satisfaction of spherical boundary conditions is that of the first odd zonal harmonics, i.e.,

$$V(r,\theta)=(Ar+B/r^2)\cos\theta. \tag{9}$$

The coordinates are shown in Fig. 1, where θ is measured from the z axis of the pore. The condition of an insulating

sphere requires that the field normal to the sphere vanish at its surface, i.e., $-\partial V/\partial r|_{r=d/2}=0$. Thus $B=d^3A/16$ and Eq. (9) becomes

$$V(r,\theta)=A\,(r+d^3/16r^2)\cos\theta. \tag{10}$$

This potential, as is well known, will have tubular current streamlines that slightly bulge around the sphere. An outline of one such surface is shown in the figure. (We calculate these surfaces below.) The point is that Eq. (10) is an exact solution for a pore of this more or less bulged shape. For the case illustrated, where the maximum diameter D_m is twice the sphere diameter d, the bulge is only 7%. With this as an exact solution for R_2, we may find a lower limit for R_1, by use of Eq. (6). This use is more accurate than the earlier use since for $d\ll D$, the cross sectional area changes slowly with distance. The resistance difference ΔR then represents an upper limit for this geometry and is also an upper limit for that of a cylindrical pore of diameter D_m since the constrictions are absent in the latter case.

To proceed to the calculations of the streamlines, we note that the total current I through any cross section of diameter D and distance z along the axis from the center of the sphere is, by Ohm's law,

$$I=\frac{2\pi}{\rho}\int^{D/2}E_z x\,dx, \tag{11}$$

where E_z is the axial component of the electric field defined by

$$E_z=\frac{-\partial V}{\partial r}\cos\theta+\frac{1}{r}\frac{\partial V}{\partial\theta}\sin\theta \tag{12}$$

and x is the distance from the central axis, while the lower limit of integration is either the surface of the sphere or zero, depending on whether $|z|$ is smaller or larger than $d/2$, respectively. The result of this calculation gives

$$I(z)=(-\pi AD^2/4\rho)\{1-[d^3/(D^2+4z^2)^{\frac{3}{2}}]\}. \tag{13}$$

In particular, for $z=0$, at the bulge,

$$I(0)=(-\pi AD_m^2/4\rho)[1-(d/D_m)^3]. \tag{14}$$

Thus we may calculate the diameter of a tube that carries constant current, i.e., a streamline, by equating the last two equations to obtain an implicit expression for $D(z)$, i.e.,

$$D^2[1-d^3/(D^2+4z^2)^{\frac{3}{2}}]=D_m^2[1-(d/D_m)^3]. \tag{15}$$

This expression is plotted in Fig. 1 for $d/D_m=0.5$. The resistance of the pore containing the sphere is obtained by use of Ohm's law with Eqs. (10) and (14), viz.,

$$R_2=[V(-L/2)-V(L/2)]/I$$
$$=\frac{4\rho L}{\pi D_m^2}\left[1+\frac{1}{2}\left(\frac{d}{L}\right)^3\right]\left[1-\left(\frac{d}{D_m}\right)^3\right]^{-1}. \tag{16}$$

TABLE I. Values of the correction factor F to be applied to the Maxwellian limit as a function of the ratio of particle to pore diameter on the basis of the upper limit theory.

$(d/D)^3$	F
0	1.0
0.1	1.14
0.2	1.32
0.3	1.55
0.4	1.87
0.5	2.31
0.6	2.99
0.7	4.15
0.8	6.50
0.9	13.7

By use of the approximation [Eq. (6)],

$$R_1=\frac{8\rho}{\pi}\int_0^{L/2}\frac{dz}{D^2}. \tag{17}$$

Substituting for D^2 from Eq. (15), we obtain

$$R_1=\frac{4\rho}{\pi D_m^2}\left[1-\left(\frac{d}{D_m}\right)^3\right]^{-1}\left[L-2\int_0^{L/2}\frac{d^3dz}{(D^2+4z^2)^{\frac{3}{2}}}\right]. \tag{18}$$

The change of resistance, as before, is given by

$$\Delta R=R_2-R_1=\frac{4\rho}{\pi D_m^2}\left[1-\left(\frac{d}{D_m}\right)^3\right]^{-1}$$
$$\times\left[\frac{d^3}{2L^2}+2\int_0^{L/2}\frac{d^3dz}{(D^2+4z^2)^{\frac{3}{2}}}\right]. \tag{19}$$

This equation, when solved in conjunction with Eq. (15) for D, gives the required upper limit. For $d\ll D_m$, $D\doteq D_m$ and we may immediately integrate Eq. (19) to obtain

$$\Delta R_{(d/D_m)\ll 1,\,(D_m/L)<1}\rightarrow\frac{4\rho d^3}{\pi D_m^4}\left[1+\frac{3}{8}\left(\frac{D_m}{L}\right)^4+\cdots\right]. \tag{20}$$

This expression has the Maxwellian value [Eq. (5)] as its limit. In addition, it is to be noted that the result is extraordinarily insensitive to the distance L over which the potential is measured. Thus the ΔR will measure quite local values of D_m. For finite values of d/D_m, we may numerically evaluate a correction term $F(d^3/D_m^3)$ defined by

$$\Delta R_{(D_m/L)\ll 1}=(4\rho d^3/\pi D_m^4)\cdot F(d^3/D_m^3). \tag{21}$$

The results of such a calculation are shown in Table I. The initial phase of $F(d^3/D^3)$ may be approximated as

$$F(d^3/D_m^3)=1+1.26_8 d^3/D_m^3+1.1_7 d^6/D_m^6. \tag{22}$$

This result, that an overestimate for the pore resistance has only terms in d^3/D^3 and higher in its corrections to the Maxwellian limit, shows that the arbitrarily corrected Gregg and Steidley form, with its terms in d^2/D^2, cannot be correct.

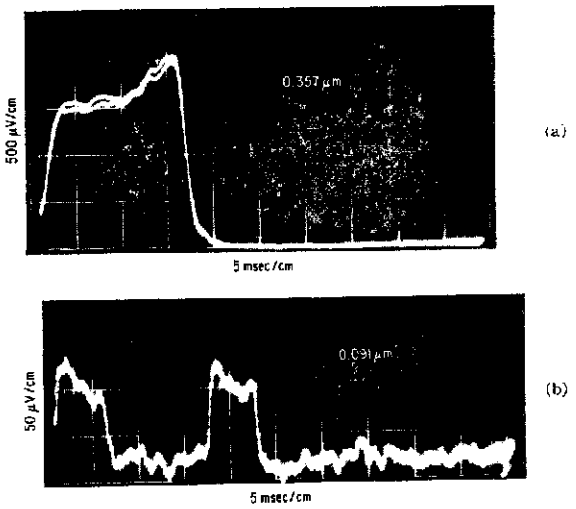

FIG. 2. (a) Double exposure of oscilloscope screen showing the signals from two polystryene spheres, each with a diameter of about 0.357 μ, on passing at zero pressure difference through a tapered pore, with end diameters of 0.49 and 0.45 μ, in a 2.9 μ thick plastic membrane. The difference in signal amplitudes represents a difference in diameters of 40 Å. The parallel wiggles reveal local undulations of about 50 Å in the pore diameter. (b) Oscilloscope trace showing the signals from two polystyrene spheres, each with a diameter of about 0.091 μ, on passing through the same pore as in Fig. 2(a), but in the opposite direction.

Lastly, we note without proof that for a sphere of finite resistance the limiting expression for the resistance increment [Eq. (5)] is modified by a factor $(2-2k)/(2+k)$, where k is the ratio of solution resistivity to sphere resistivity. This agrees with the results of Maxwell and Lord Rayleigh.

In summary, this calculation complements that of Kubitschek and Gregg and Steidley in giving an upper bound for the resistive pulse and one which agrees with the Maxwellian limit. In addition, this calculation allows specific consideration of the effects of pore length and finite resistivity of the sphere.

EXPERIMENTAL PROCEDURES

Pores are produced by etching 10 μ thick sheets of Lexan or Makrofol that have been irradiated with collimated fission fragments from ^{252}Cf to a density of about 5×10^3/cm^2. (The range of the fragments in the plastic is about 18 μ.) The etching is typically performed at 70°C in a solution of 3.12N NaOH with $\frac{1}{2}$vol% of Benax 2Al solution (Dow Chemical Company, Midland, Michigan) added.[10] The latter is an anionic surfactant used to insure even wetting of the pores. Under the conditions outlined above, the pore increases in diameter at a rate of approximately 100 Å/min. After a suitable time to give the desired pore size, the sample is rinsed in water and 5% acetic acid. One can observe individual pores as small as 0.1 μ in diameter in a 10 μ thick sheet under a 200 power stereomicroscope with dark field illumination. A small square is cut out and mounted with epoxy over a hole about $\frac{1}{3}$ mm in diameter in a plastic disk that forms the partition between the two chambers containing the electrolyte. There will generally be several pores in this area. These are observed under the stereomicroscope and the plastic square is moved around on the epoxy-coated surface of the disk with a tweezer tip while the epoxy is still fluid until all but one pore are sealed. The pore may be thinned by stripping off several layers of the plastic with fine pointed tweezers after it is bonded to the plastic disk. The manufacturing process for Makrofol gives it a directional, layered structure. Microtoming might also work. Shortening the pore significantly reduces the intrinsic noise level if its resistance is many megohms.

The disk is mounted with O-rings between two Plexiglas chambers, each about 1 cm^3 in volume, fitted with silver–silver chloride electrodes. A solution of 0.1N KCl is prepared with a few drops per 100 ml of Tween 60, a nonionic surfactant, to prevent later particle coagulation and pore plugging (Atlas Chemical Industries, Incorporated, Wilmington, Delaware). In addition, this solution is filtered on injection into the cell through a filter membrane, e.g., MF-Millipore, type VS, nominal pore size, 250 Å (Millipore Corporation, Bedford, Massachusetts). The resistance across the membrane is then measured. Ultrasonic agitation of the cell may be needed to establish electrical continuity through the pore. If the cell resistance R_m is several megohms or tens of megohms, the measuring circuit may conveniently consist of R_m in series with the 1 MΩ (or if modified, several megohm) internal resistance R_L of an oscilloscope and with a battery, perhaps with rheostatic control, to provide a voltage gradient across the pore of several thousand volts per centimeter. All units are contained in shielding boxes. An ammeter and a voltmeter, e.g., Keithley 610C electrometers, are added as needed. The noise level of the oscilloscope trace and the particle content of the filtered solution are checked before the test particles are added (or checked by flow from the chamber not containing the test particles). These particles are polystyrene latex spheres of closely uniform size (Dow Chemical Company, Midland, Michigan). We report here measurements on six particle sizes ranging from 0.091 to 0.357 μ. To charge the cell we add approximately 1 μl of the original 10% suspension of spheres in water.

Plugging of the aperture is a chronic problem with particle counters. We have observed the aperture of an operating cell under a microscope and find that plugging with polystyrene spheres may occur owing to interactions, presumably both electrostatic and kinetic, between the particles and the edge of the aperture, and between the incoming particles and those already stuck to the aperture, even though all particles are smaller than the aperture. We have relieved the plugging problem for polystyrene latex particles in KCl solution by the use of the nonionic surfactant and by the application of ultrasound to the cell

when, on occasion, it is needed.[11] The data below were gathered over a period of three weeks before ultrasonic cleaning finally split the plastic membrane.

EXPERIMENTAL RESULTS

Figure 2(a) is a double exposure of the oscilloscope signals from two consecutive polystyrene spheres, of nominal diameter 0.357 μ, passing through a tapered pore calculated to have an effective length of 3.3 μ and end diameters of 0.49 and 0.45 μ. The voltage across the cell was 0.343 V and the current was 0.236×10^{-7} A. The pressure difference was less than 0.2 mm H_2O. The particles moved, as they do in all experiments reported here, as if they had a negative charge. It is interesting to note that, while these particles give closely identical voltage traces, one is on the average about 4% higher than the other. Employing our empirical formula for the dependence of signal on particle size [Eq. (23)], we find that this difference corresponds to a difference of only 40 Å in particle size. (The stated standard deviation of these spheres in 56 Å.) It is apparent from the low noise level that this difference could be determined more accurately by further amplification of the signals. In addition, the parallel wiggles in the traces show small undulations in the pore diameter superimposed on the general taper of 0.02 μ over the length.

Figure 2(b) is a single trace showing the consecutive passage through the same pore [in the opposite direction from Fig. 2(a)] of two 0.091 μ spheres. In this case, the voltage across the cell is 1.28 V, the current is 0.920×10^{-7} A, and the pressure difference is about zero.

The time of passage is seen to be approximately one third of that in Fig. 2(a), which corresponds roughly to the

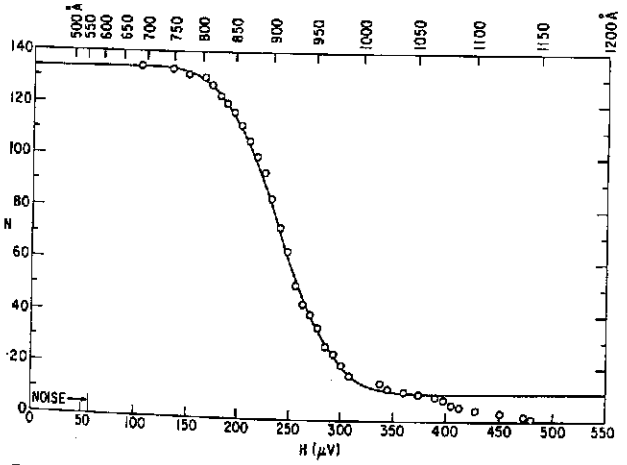

FIG. 3. Particle size distribution for a sample of 134 spheres of mean diameter 910 Å. The vertical axis plots the number of spheres whose pulse amplitudes are equal to or greater than H, the lower abcissa. The equivalent particle diameter (Å) is shown on the upper abcissa. The solid line is a standard deviation curve ($\Delta d = 54$ Å) for the 124 pulses whose amplitudes are less than 360 μV. The last 10 pulses are presumed to be doublets.

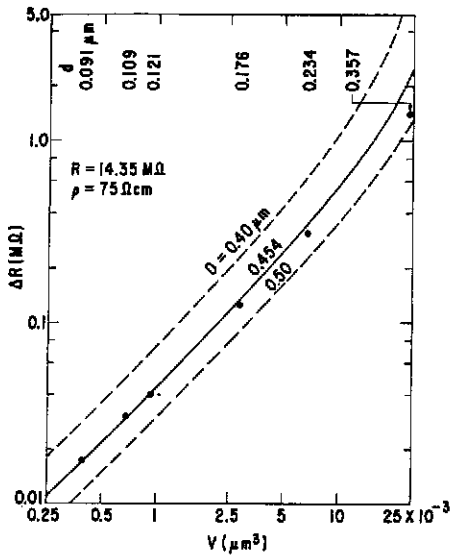

FIG. 4. Plot of resistive pulse ΔR vs sphere volume for six sizes of polystyrene spheres. The diameters are shown at the top. The solid line is the theoretical upper limit curve, for pore diameter $D = 0.454\,\mu$, that best matches the data at small volumes. The sensitivity of ΔR to changes in pore diameter is indicated by the dashed curves calculated for $D = 0.40$ and $0.50\,\mu$.

fact that the electrophoretic and electro-osmotic velocities are about three times greater, owing to the increased voltage for this case.

Figure 3 is a plot for 134 pulses from nominally 0.091 μ spheres of the number N with peak heights equal to or greater than H microvolts, vs H. The solid line shows a theoretical standard deviation curve drawn to fit the data for 124 pulses up to $H = 360\,\mu$V. The upper 10 pulses are presumed to be double particles. (There were eight further pulses with $H > 480\,\mu$V that were not recorded.) The claimed standard deviation for the particles is 0.0059 μ. If the mean diameter is taken to be 0.091 μ from the Dow standardization, our results give a standard deviation of 0.0054 μ. The abscissa at the top shows the particle diameters in angstroms. The smallest observed particle is 690 Å. We judge that particles 600 Å (0.060 μ) in diameter would still be distinguishable from background noise. [The noise level in Fig. 3 is higher than in Fig. 2(b) because an amplifier with higher impedance was used.] One may also note from the upper abscissa, plotted from Eq. (5), that the sensitivity to changes in diameter increases with increasing diameter, specifically, as the square of the diameter, or even more rapidly as the diameter of the sphere approaches that of the pore.

The average pulse heights for monodisperse polystyrene spheres of six different sizes at a pressure difference near zero have been converted to changes of resistance ΔR and plotted vs sphere volume in Fig. 4. The diameters of the particles are shown at the top.[12] The three curves are plots of Eq. (21) for pore diameters of 0.40, 0.454, and 0.50 μ, respectively, and for electrolyte resistivity $\rho = 75\ \Omega\cdot$cm. The

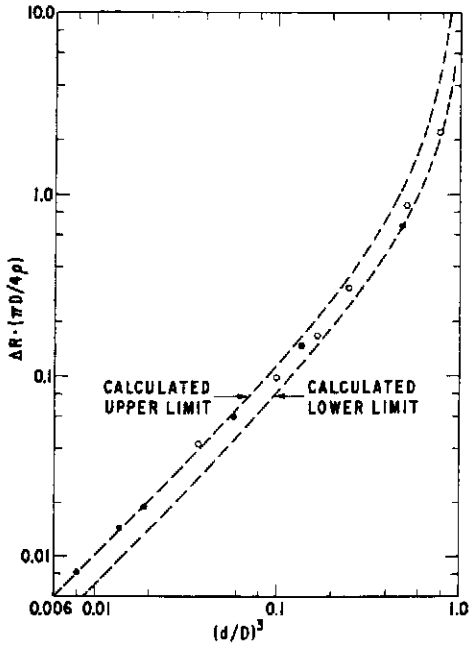

FIG. 5. Dimensionless plot of the resistive pulses as a function of particle size. The resistive pulse is normalized in terms of the resistance of a circular cylinder of diameter and length D, where D is the pore diameter. The particle diameter is normalized to the pore diameter. The data are those of Fig. 4 (filled circles) and for model experiments by Gregg and Steidley (open circles). The upper theoretical curve is that of Eq. (21) while the lower is that of Gregg and Steidley. As expected, the data approach these limits in the extremes of small and large particles, respectively. ●—Present data, ○—model experiments by Gregg and Steidley.

value 0.454 μ was determined as the best fit for the data. As expected, the data points to the right fall below the theoretical curve for the upper limit of ΔR. The data best fit a semiempirical equation

$$\Delta R = (4\rho d^3/\pi D^4)[1+0.73(d^3/D^3)]. \tag{23}$$

In the introduction to this section we gave numbers for the pore geometry. We derived these from the data shown previously. The ratio of pulse heights for 0.091 μ spheres at the narrow and wide ends of the pore is about 1.37. Since ΔR varies inversely as the fourth power of the pore diameter for small volumes, the pore diameter at the wide end is 0.49 μ. The smaller diameter is on the original surface of the stripped sheet. With a pore resistance of 14.35 MΩ and an electrolyte resistivity ρ of 75 Ω·cm, this yields, with the use of Eq. (6), an effective pore length L of 3.3 μ, or a corrected geometrical length of 2.9 μ. Attempts to locate and measure the diameters of the pore by electron and scanning electron microscopy were unsuccessful.

In Fig. 5 we plot the experimental data of Fig. 4 in dimensionless form, along with Eqs. (21) and (15), as cast in the same dimensionless coordinates. We also include data from model measurements by Gregg and Steidley.[3] The data, as previously noted, are expected to lie on the upper curve for small relative volumes and to approach the Kubitschek–Gregg and Steidley limit or lower curve for large relative volumes. This trend is clearly seen and, further, both sets of data are consistent with one another.

DISCUSSION

The data and considerations presented here give further evidence for the power of the resistive pulse method for characterizing particles in solution and, in addition, move the capabilities well into the colloidal size range. The striking sensitivities of response on particle size suggest that this technique can be used to study the adsorption of large molecules, such as proteins, on solid surfaces. There is also the possibility of tracing the detailed internal geometry of a pore by virtue of the D^{-4} dependence of the signal. As far as we are aware, there is no other technique of comparable power and sensitivity. As mentioned in the introduction, the particles are transported by a sum of electrophoretic and electro-osmotic effects. The effective mobilities are about 2×10^{-5} cm²/V·sec for the data reported here. It is clear, on general grounds, that the electro-osmotic effects can be independently measured (by streaming potential, for instance), and, hence, an absolute value for electrophoretic mobility obtained. As indicated earlier, we propose to make this point the subject of a later communication.

The question presents itself, what is the ultimate sensitivity of a detector of this type? To approach this problem, we consider a simple series circuit consisting of a voltage source, a membrane with a single pore of diameter D and resistance R_m, and a load resistance R_L. If ΔR_m is the change of resistance of the pore on insertion of a particle of diameter $d \ll D$, then

$$\Delta E_m = -\Delta E_L = E_m \Delta R_m/(1+\alpha)R_m, \tag{24}$$

where ΔE_m and ΔE_L are the corresponding voltage changes across the membrane and load resistance, respectively, E_m is the voltage across the membrane, and $\alpha \equiv R_m/R_L$. With Eqs. (2) and (5) we obtain

$$\Delta E_m = -\Delta E_L = \frac{1}{(1+\alpha)} \frac{E_m}{L} \frac{d^3}{D^2}, \tag{25}$$

where L is the length of the pore. This voltage is maximized by making the load resistance large, i.e., going towards a condition of constant current where $\alpha \ll 1$. The ultimate limit in resolution is determined by Johnson noise in the source resistance, which in the case assumed is essentially equal to the membrane resistance R_m. This noise, for the general case, is given in rms volts by

$$E_N = 2(kTR\Delta f)^{\frac{1}{2}}, \tag{26}$$

where k is Boltzmann's constant, T the absolute temperature, R the source resistance, and Δf the bandwidth of the measurement. For $R = 15$ MΩ, $\Delta f = 10$ kHz, and $T = 300$ K, we obtain $E_N = 47$ μV. In practice, the amplifier also adds

noise, which should be added in quadrature to the Johnson noise.

As an example of what would appear to be feasible in the near future by current techniques, let us assume $R_m = 15$ MΩ (corresponding to $D = 2500$ Å, $L = 10\,000$ Å, and $\rho = 73.5\ \Omega\cdot$cm), $\alpha \ll 1$ (constant current approximation), a field gradient of 10^4 V/cm, and a total noise level of 250 μV from a high impedance amplifier over the membrane. Then Eq. (25) gives a minimum detectable particle diameter of 250 Å. By further developments in technique one might possibly go to about 150 Å by letting $D = 1000$ Å, $L = 2000$ Å, reducing ρ, and increasing the field gradient. For the short particle transit times implied by this small thickness and high voltage gradient, the capacitance of the membrane can become a problem in limiting the effective bandwidth of the detector. In this instance a negative capacitance electrometer (e.g., Keithley model 605) would be helpful.

In addition to the Johnson noise and amplifier noise, there is a noise that depends on the voltage level across the membrane whose origin we do not understand. From Eq. (25), the signal would be increased by increasing the voltage across the membrane. One limit, of course, is that of dielectric breakdown of either the membrane material or the aqueous channel. This limit is rather high in that the dielectric strength of polycarbonate,[13] for instance, is on the order of 1.4×10^5 V/cm, while that for pure water[14] is about 10^6 V/cm. Another limit, generally lower, is that occasioned by heating of the liquid column. While this heating is difficult to calculate exactly, it is simple to calculate an upper limit by assuming that the membrane material is perfectly insulating. The problem becomes that of calculating the temperature distribution along a current carrying wire under the condition that the surface is impervious to heat. This problem was solved many years ago by Kohlrausch[15] and results in a parabolic distribution of temperature with a maximum at the middle of the pore

$$\Delta T_{max} = 0.239 E_m^2/8\rho K, \qquad (27)$$

where ΔT is the temperature increment in degrees centigrade over that of the ends, E_m is the voltage across the membrane, ρ is the resistivity of the solution, and K is the thermal conductivity of the solution. It is interesting to note that this result does not depend on the length of the pore. If we use[16] $K = 1.4_4\times10^{-3}$ cal/cm·sec·deg and $\rho = 75\ \Omega\cdot$cm together with the condition of the experiment shown in Fig. 2(b) ($E_m = 1.28$ V), the ΔT_{max} is approximately 0.35°C. Hence, heating is not presumed to be important in this case, although small increases in membrane potential gave significant increases in noise level. It seems probable that this noise bears some analogy to the excess noise of current carrying semiconductors,[17] but the unraveling of its cause and possible cure must await further experimentation.

Since this manuscript has been completed, an interesting pair of papers on "Electrical Sizing of Particles in Suspensions" has been published [N. B. Grover, J. Naaman, S. Ben-Sasson, F. Doljanksi, and E. Nadav, Biophys. J. **9**, 1398, 1415 (1969)]. These workers give careful consideration of the hydrodynamic and electrical conditions at the entrances to orifices and report measurements on large ($\sim 3\,\mu$) polystyrene spheres as well as pollen passing through large apertures ($D \geq 100\ \mu$). The results of the measurements on the artificial spheres verified the Maxwellian factor of $\frac{3}{2}$ to better than 2%. For estimation of the effect of finite particle size, however, the authors use an adaptation of Maxwell's formula which in our notation becomes

$$\Delta R = (4\rho d^3/\pi D^4)(1 + 2d^3/3D^2L + (2d^3/3D^2L)^2 + \cdots),$$

where L is the effective length of the pore. This expression differs from those we propose in the inclusion of L. It indicates, therefore, that any correction will disappear for a sufficiently long pore. This point of view is at variance with ours. Fortunately, in their applications, the correction amounts to less than 2% and so is not important.

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[12] The spheres shown as 0.121 μ were labeled as 0.126 μ, which would correspond to the small datum point at $1.05\times10^{-3}\ \mu^3$ and 0.0397 MΩ. Since this is off the curve for the other data, these spheres were com-

pared by electron microscopy with those labeled as 0.109 μ diam and were corrected to 0.121 μ on the assumption that the value 0.109 μ was correct.

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Generating Cold Gas for Photomultiplier Cooling

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The somewhat obvious but relatively unused technique for generating cold gas by bubbling room temperature N_2 directly into liquid nitrogen offers twice the cooling time of the conventional immersed heater or continuous gas flowthrough systems. An apparatus for implementing the technique is illustrated.

BECAUSE of several temperature dependent factors,[1] including thermionic emission, photosensitivity, and spectral response, many photomultipliers sensitive in the near infrared give best[2] over-all performance at some temperature between 0 and −50°C. Cold gas, about −195°C at the generating point to allow for losses, is a suitable refrigerant for this range; it is commonly generated by a heater immersed in a Dewar of liquid nitrogen (LN), or by flowing dry N_2 gas through a coil immersed in LN.

A method of cold gas generation which extracts greater cooling power from the LN is to bubble N_2 gas directly into LN. The thermodynamic calculations summarized in Table I suggest that the volume of cold gas obtained using this technique is about twice that of the other methods for a given mass of LN. Thus, the nuisances of too frequent LN replenishment and condensables blocking the cooling coil in the continuous flowthrough system are overcome.

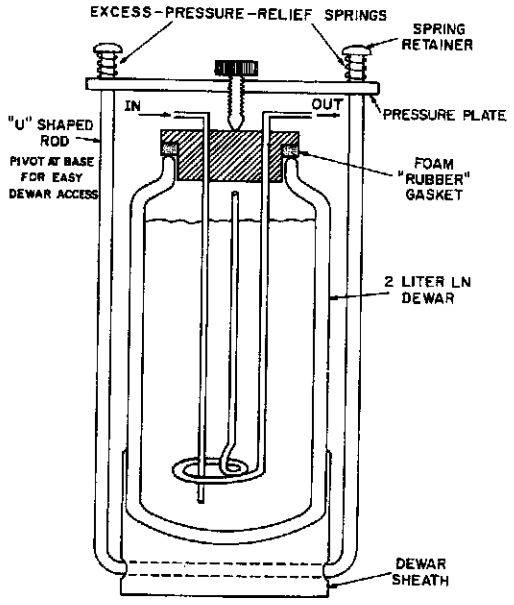

FIG. 1. Typical apparatus for utilizing bubbling gas technique for generating cold N_2 gas.

TABLE I. LN efficiencies of cold-gas generating systems. m = Mass of liquid nitrogen, ρ = density of N_2 gas at −195°C, H_{vap} = heat of vaporization of LN = 47.6 cal/g, C_p = specific heat of N_2 gas = 0.25 cal/g/°C, 220 = room temperature − LN temperature (°C).

System	Generated volume of N_2 gas at −195°C	Relative operating time or volume
Immersed heater	m/ρ	1.0
Continuous flowthrough	$(m/\rho)(H_{vap}/220\,C_p)$	0.9
Bubbling gas	$(m/\rho)(1+H_{vap}/220C_p)$	1.9

The arrangement shown in the diagram has proven very convenient in laboratory use. Photomultiplier temperature is easily adjusted by controlling input gas flow rate. We obtain about 7 h continuous operation at −25°C from a single 2 liter charge of LN: Cold gas flow is through 0.75 m of Styrofoam insulated tubing to a large end-on type photomultiplier (RCA C31000F) housed in 1.5 cm thick Styrofoam with a 3 mm thick glass window. About 1000 liters (35 cu ft) of N_2 gas are used per 2 liters of LN. As with any photomultiplier cooling technique, care should be taken to insure uniform gradual refrigeration of the entire tube.

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