THE APPLICATION OF THE COULTER COUNTER TO PROBLEMS IN THE SIZE ANALYSIS OF INSOLUBLE DRUGS

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Received March 21, 1963

A method of programming an electronic computer has been developed which fits the results of size analyses obtained by means of a Coulter counter to a logarithmic normal curve from which a specific surface area figure is derived. Applications of the method to suspensions of phenothiazine are discussed.

The Coulter counter described by Kubitschek (1960) offers a new technique of counting solid particles in the sub-sieve range, which in common with automatic "flying spot" microscopes, such as those introduced by Dell (1954), Hawksley (1954) and Roberts and Young (1951), has two important advantages over classical methods of microscopy; that of greatly reducing the time taken to complete a size distribution, and the removal of operator bias. Recent developments in optical microscopy, such as the double-image micrometers of Timbrell described by Barnett and Timbrell (1962) and Cooke, Troughton and Simms described by Dyson (1960) have greatly reduced operator bias by increasing the precision with which the statistical diameters are measured. These methods still leave the operator with the job of locating and sizing the particles under a bench microscope, and the time involved is considerable.

The Coulter counter is well suited for sizing particles between 1 μ and 76 μ in diameter. This size range adequately covers most of the finely-divided drugs such as phenothiazine (*see* Kingsbury, 1958), and griseofulvin (*see* Atkinson, Bedford, Child and Tomich, 1962), where it has been proved that particle size has a biological effect.

Information fed to the "Mercury" Computer

The Coulter counter sizes relatively non-conducting particles according to changes in the resistivity of an electrolyte solution in which they are suspended. The electrolyte is made to flow through a small diameter (30 μ and upwards) cylindrical aperture in the wall of a glass vessel and the passage of a particle creates an impulse proportional to its size which is amplified in an electronic circuit.

To use the services of a "Mercury" electronic computer to obtain the data required, certain changes in the method of calculating the results were made. To make these intelligible, the functioning of the counter is briefly discussed.

When performing a size determination on a sample, the instrument, by means of two controls, is set to count all particles with diameters greater than a certain threshold value. The first, the threshold control, is a potentiometer with a continuous scale graduated from 0 to 100 whose readings is denoted by t', and the second, the current selector switch,

has ten settings numbered from 1 to 10, denoted by I. The aperture resistance, Ω, has to be determined for the supporting electrolyte used and this is done directly with a good DC voltmeter. From the I value and the aperture resistance, a "scaling factor", F, can be derived from tables supplied with the instrument. This, together with a calibration factor, k, translates the dial settings into particle diameters, d, according to the expression

$$d = k\sqrt[3]{t} \qquad \text{where } t = t'F$$

It should be noted that t has the dimensions of volume, which, for particles of equal density, is proportional to weight, and hence the t values are also used to transform the numbers of particles counted into weight fractions.

It would have been cumbersome to store the full table of F values in the computer, so a linear approximation was derived. Using this for a particular current setting I and aperture resistance Ω, the F value is given by

$$F = a_I + b_I \frac{(\Omega - 30{,}000)}{5{,}000}$$

The constants for each of the ten settings of I are given in Table I.

TABLE I

CONSTANTS FOR EACH OF 1 TO 10 SETTINGS OF I

Setting I	a_I	b_I
1	1·0	0
2	0·5014	0·00022
3	0·2521	0·00026
4	0·12744	0·00030
5	0·06515	0·000336
6	0·03401	0·000348
7	0·018481	0·0003649
8	0·010818	0·0003960
9	0·007178	0·0004551
10	0·005743	0·0005722

For Ω in the range 15 to 40 K ohms the values given by this approximation have a maximum error (when compared with the tabulated values) of about 0·5 per cent which occurs for setting 10: for I = 1 up to 9, the maximum error is 0·1 per cent. At present, this seems adequate but only a slight amount of work would be required to derive a quadratic approximation which fitted the tabulated values even more closely.

When the t values have been derived, a value has to be assigned to the calibration factor k, so that the particle diameters may be calculated. This factor can best be obtained by direct calibration with mono-disperse spheres of a diameter calculated by other means, and the value of k supplied by the manufacturers has been used for the experiments to be described. There is a further variable control on the instrument, the gain selector rheostat, which has six positions and gives pulse height increase in a $\sqrt{2}$ progression which affects the calibration factor (k) proportionately. This control, therefore, offers a means of varying the size range

covered by the machine without changing the diameter of the aperture or the conductivity of the supporting electrolyte.

In practice, when using the counter for routine control of a single product, a regular pattern of values of t′ and I, which is known by experience to give a reasonable change of particle diameter, is used, and once the particle diameters have been determined for each setting of the control knobs, these are used for each size analysis without the need for further calculation. However, in the computer programme, the calculation is done automatically on every occasion and any independent variation of t′ and I can be used with equal facility. If, as a result of making such variations, the particle diameters in descending order of magnitude are not known, this is of no importance, since the computer automatically sorts out the values into the correct order before proceeding with the calculation.

It is usual to calculate 12 to 16 values of d, each representing lower limits of size above which total particle counts are obtained experimentally. The counting is done automatically and the total number of particles larger than each particular value of d is recorded on the instrument. There are, however, corrections for coincidence, that is the counting of two or more particles as a single particle, and for background count, which have to be applied to the mean count $\bar{n}'$. The equation for calculating the coincidence factor, P, is given as

$$P = 2{\cdot}5\,(D/100)^3\,(500/V)$$

where D is the aperture diameter in microns and V is the volume of suspension in which the particles are counted, in microlitres. P is therefore an instrumental constant and its relationship to n″, the correction to be added to the number of particles to allow for coincidence, is

$$n'' = P(\bar{n}'/1{,}000)^2$$

The background counts, b, are determined experimentally at each dial setting, using the supporting electrolyte only, and the corrected expression for the total number of particles n, above any particular diameter, d, is

$$n = \bar{n}' + P(\bar{n}'/1{,}000)^2 - b$$

All the information required by the computer before it can perform the necessary calculation has now been briefly discussed. The data it requires are: The coincidence factor, P; the calibration factor, k; the aperture resistance, Ω, and, for each setting of the controls: the threshold control setting, t′; the current selector switch setting, I; the mean count, $\bar{n}'$; the background count, b.

Information Printed Out by the "Mercury" Computer

The actual number of particles, Δn, in each size range bounded by successive values of d, is obtained by subtracting adjacent n values. The mean volume factor t is the arithmetic mean of adjacent t values and hence $\Delta\bar{n}t$ gives the contribution to the total weight of each size fraction.

To calculate the weight percentage of particles in each size range, a figure representing the total weight of particles in the sample must be derived. This is a difficulty common to all counting methods of size analysis, because the contribution to the total weight of particles smaller in diameter than the smallest particle which has been counted, is unknown. It is usually possible by experience and inspection of the weights of sample in each size fraction to form a "reasonable" estimate of the total weight and in many cases, it may be assumed that the weight of particles below the smallest size counted is negligible. Such assumptions, however, need not be made if the distribution can be shown to obey a mathematical

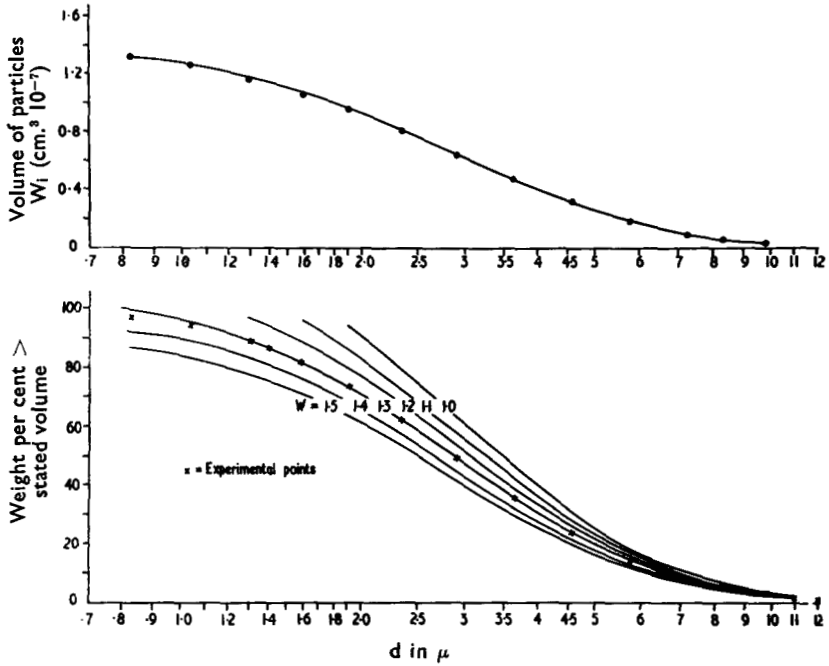

FIG. 1. (*a*) Upper figure, data obtained by summation of particle volumes given in the last column of Table III plotted against particle diameters in fourth column of Table II. (*b*) Experimental weight percentages from Table II, columns 4 and 5. Theoretical distributions using different values of W in the relationship $P = (w_1/W)$.

relationship. Several years' experience of determining particle size distribution by various methods has demonstrated that, for milled insoluble drugs, the distributions follow closely a logarithmic normal law (Thornton, 1959). This fact has now been used in programming the computer to obtain a complete size distribution. The equation for a logarithmic normal distribution may be written as:

$$\mathbf{P} = \int_d^\infty \left\{ \frac{1}{\sqrt{2\pi}\,\ln\alpha}\,\frac{1}{u}\exp\left[\frac{-(\ln u - \ln d_w)^2}{2(\ln\alpha)^2}\right]\right\} du$$

where **P** is the percentage by weight of spherical particles with diameters

greater than d, d_w is the weight mean diameter, i.e. the diameter of a hypothetical sphere such that 50 per cent by weight of the particles have larger diameters and 50 per cent have smaller diameters, and α is the standard deviation.

Fig. 1a shows results on the sample of phenothiazine suspension discussed later, and Fig. 1b shows the w_1 values of Fig. 1a scaled down by different factors (1/W). The basis of the method is to choose a particular value of W which best fits a logarithmic normal curve so that $\mathbf{P} = w_1/W$ and this is obtained by the method of least squares according to the following argument. The curve is fixed when $\ln d_w$ and $\ln \alpha$ are known, and it is convenient to work in terms of two constants derived from these, viz.

$$x = -\ln d_w/\ln\alpha$$

and

$$c = 1/\ln\alpha$$

The value of **P** for a particular value of d is denoted by $\mathbf{P}(x + c \log d)$; it may be seen that

$$\mathbf{P}(x + c \log d) = \int_d^\infty \frac{c}{\sqrt{2\pi}} \cdot \frac{1}{u} \exp - [\tfrac{1}{2}(x + c \log u)^2]du$$

Suppose that there are n "reference diameters" $d_1, d_2, \ldots d_n$ with associated results, $w_1, w_2, \ldots . w_n$.

The sum of squares of deviations is then

$$\left\{\frac{w_1}{W} - \mathbf{P}(x + c \log d_1)\right\}^2 + \left\{\frac{w_2}{W} - \mathbf{P}(x + c \log d_2)\right\}^2 + \\ + \ldots \left\{\frac{w_n}{W} - \mathbf{P}(x + c \log d_n)\right\}^2$$

which is denoted by

$$S = \sum_{i=1}^{n} \left\{\frac{w_1}{W} - \mathbf{P}(x + c \log d_1)\right\}^2$$

The aim is to choose W, x and c so that S is minimised, i.e. so that the curve fits the experimental points in the "best" manner.

It is not possible to write down explicit expressions for the values of W, x and c which do this, so an iterative method must be used. Initial approximations for W, x and c are obtained and corrections to these are calculated by the Newton-Raphson method: suppose these are ΔW, Δx, and Δc. The usual procedure would be to take $W + \Delta W$, $x + \Delta x$, and $c + \Delta c$ as better approximations, calculate corrections to these, and repeat until the approximations were sufficiently close to the true answer. However, in this case, this procedure has been modified to speed up the calculation.

The modification is to calculate the values of S for the following set of values

$$(W - \Delta W, x - \Delta x, c - \Delta c)$$
$$(W \quad , x \quad , c \quad)$$
$$(W + \Delta W, x + \Delta x, c + \Delta c)$$

and continuing with

$$(W + 2\Delta W,\ x + 2\Delta x,\ c + 2\Delta c)$$

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up to $(W + 20\Delta W,\ x + 20\Delta x,\ c + 20\Delta c)$ if necessary, in order to find three values which straddle the minimum. Having found three values, a quadratic curve is fitted to the three corresponding values of S and the point $(W + \lambda\Delta W,\ x + \lambda\Delta x,\ c + \lambda\Delta c)$ determined which minimises this quadratic function. This is then used as the next approximation to the solution.

The computer stops this process when the solution is "sufficiently" accurate, this being tested in the following way.

At each iteration $\frac{\Delta W}{W}$, $\frac{\Delta x}{x}$ and $\frac{\Delta c}{c}$ give the fractional corrections to the three "unknowns". The computer takes these three quantities with positive signs, adds them and stops the iteration procedure when the sum is less than 0·00001. This insures that the last correction made to each of the unknowns is less than 0·001 per cent and this will also represent the accuracy of the unknowns (or the maximum inaccuracy).

The relationship between the two parameters of a logarithmic normal weight distribution which serve to define it (namely the weight mean particle size, d_w, and the standard deviation, α) and the surface mean diameter, d_s, which is required to calculate the specific surface area, is obtained from the Hatch-Choate relationship (Herdan, 1960)

$$\frac{2 \ln d_s}{3 \ln d_w} = \frac{2 \ln M + 2 \ln^2\alpha}{3 \ln M + 4{\cdot}5 \ln^2\alpha}$$

M is the geometric mean diameter of a frequency distribution and α is, by definition, the ratio of the diameter above which 84·13 per cent of the distribution occurs, to the corresponding 50 per cent diameter. These percentages may be either by weight or by number.

From the above expression,

$$\log d_s = \log d_w - 1{\cdot}151 \log^2 \alpha$$

The specific surface area of spherical particles having the calculated distribution is then

$$S.A \times p = 6 \times 10^4/d_s$$

where $S.A$ is the surface area in cm.²/g., p is the absolute density of the drug in question, and d_s is the surface mean diameter in microns.

This calculation is easily fed to the computer, which finally prints out the following data for each setting of the controls: The threshold control setting, t′. The current selector switch setting, I. The true count, n. The particle diameter, d. The cumulative weight greater than each value of d, w_1/W.

The corrected cumulative weight derived from the log normal curve, and, for the entire distribution:

$$\text{The value of } S.A \times \text{p in cm.}^2/\text{cm.}^3$$

EXPERIMENTAL

The experiments made to test the performance of the Coulter counter and the usefulness of this computer programme were on samples of commercial suspension containing 50 per cent w/v of phenothiazine.

The particles were between 1 and 10 μ in diameter, hence an instrument tube with a 50 μ aperture, and a nominal sample volume of 0·05 ml., were used. The t′ and I values and the gain index, were selected to give sixteen calculated values of particle diameter between 0·82 and 12·3 μ and the dilution was such that about 35,000 particles greater than 0·82 μ were counted. The information printed out by the computer on a typical suspension is shown in Table II.

TABLE II

SIZE ANALYSIS OF PHENOTHIAZINE SUSPENSION

(Data obtained by means of the Coulter counter printed out by the "Mercury" computer)

Instrument controls		Total particles above corresponding value of d	Particle diameter in μ	Cumulative weight per cent greater than d	
t′	I	n	d	Experimental	From log normal curve
100	1	−0·5	12·30	0·00	0·96
70	1	2·0	10·92	1·53	1·57
50	1	3·5	9·76	2·18	2·43
30	1	13·0	8·23	4·91	4·46
20	1	21·0	7·19	6·35	6·89
20	2	82·5	5·72	13·00	13·21
20	3	263·2	4·55	22·80	22·65
20	4	727·7	3·62	35·49	34·91
20	5	1659·7	2·90	48·43	48·75
20	6	3601·7	2·34	62·35	62·29
20	7	6602·9	1·91	73·77	73·73
20	8	10606·2	1·60	82·34	81·99
20	9	14130·3	1·40	87·04	86·97
20	10	15171·3	1·31	88·05	89·11
10	10	25262·2	1·04	94·68	94·54
5	10	35091·3	0·83	97·91	97·56

(Specific Surface Area) × (Density) = 25,690 cm.²/cm³.

In obtaining a particle size distribution by any counting technique one fundamental assumption must be made. Generally speaking an estimate is made of the number or weight of particles too small to have been included, or these are neglected altogether and the number or weight counted is assumed to represent adequately the entire sample. The assumption made here is that the distribution is logarithmic normal.

It is, however, theoretically possible to account for the weight of phenothiazine used by the Coulter counter for each count, by direct weighing of the original sample and use of accurate methods of dilution of the suspensions. For these experiments, an electrolyte containing 0·9 per cent NaCl and 0·01 per cent Perminal BXN (an anionic wetting

agent) was used for making the dilutions which finally contained approximately $2{\cdot}5 \times 10^{-7}$ g. of phenothiazine in 0·05 ml. of suspension. The method of obtaining a total weight figure using the relevant data from Table II is shown in Table III, where the last column gives the total volume of solids in each size range assuming the particles are spherical. The total weight of phenothiazine is then calculated to be $1{\cdot}8 \times 10^{-7}$ g.

TABLE III

ESTIMATED WEIGHT OF PHENOTHIAZINE IN 0·05 ml. OF DILUTED SUSPENSION CONTAINING A CALCULATED QUANTITY OF $2{\cdot}5 \times 10^{-7}$ g., USING THE SIZE ANALYSIS DATA OF TABLE II

Number of particles between successive values of d N	Mean diameter in each size range (μ) $\bar{d}$	Volume of a sphere of diameter $\bar{d}$ ($cm.^3 \times 10^{12}$) V	Total volume in each size range ($cm.^3 \times 10^{12}$) N.V
2·5	11·6	817·5	2,044
1·5	10·3	572·2	858
9·5	9·0	382	3,630
8·0	7·71	240	1,920
61·5	6·46	140	8,610
180·7	5·13	72	13,010
464·5	4·08	37	17,190
932·0	3·26	18·1	16,870
1,942·0	2·62	8·5	16,500
3,001·2	2·12	4·9	14,700
4,004·3	1·75	3·0	12,012
3,524·1	1·50	2·0	7,048
1,041·0	1·36	1·45	1,509
10,090·9	1·17	1·0	10,090
9,829·1	0·93	0·5	4,915
—	<0·82	—	Say 4,094
			$\Sigma = 135{,}000 \times 10^{-12}$ cm.^{3}

$\therefore$ Weight of phenothiazine (density 1·36) = $1{\cdot}84 \times 10^{-7}$ g.

The fact that the weights of phenothiazine do not agree more closely can be ascribed to a number of factors. Perhaps the more important of these relate to the instrumental constants. The calibration factor, k, was that given by the makers since our initial experiments showed that a comparison of size analyses by Andreasen pipette, which was our standard method but was only applicable to the top size ranges, and the Coulter counter, were in excellent agreement. Also the volume of suspension used for each count is nominally 0·05 ml. but since this does not affect the performance or calibration of the instrument an absolute value has never been obtained. However, departure from this figure would materially affect calculations, e.g. if the volume were 0·04 ml. the weight of phenothiazine used in each count would be $2{\cdot}0 \times 10^{-7}$ g. Other points are that the particles are not spherical and hence there will be an effect caused by unknown shape factors, and that no solid can be absolutely insoluble in the suspending liquid. On this last point, however, no difference has been detected in size analyses between dilutions of phenothiazine in the electrolyte alone and in a similar solution previously saturated with phenothiazine. This is not the case with many so-called insoluble drugs, however, and the effect of solubility of the smallest particles must be carefully watched.

Whatever the reasons may be, the direct method of estimating the total weight of solid in the sample being counted would introduce serious

errors if it were used as the basis of the calculated size distribution. The effects of three different total weights, estimated by experience, and not by the method of direct weighing, used in compiling Table III are shown in the logarithmic probability graph (Fig. 2). This method of graphical representation of a size distribution as a straight line exaggerates departures from linearity at the "tails" of a size distribution but the effect is nevertheless real and will be more marked when the particles are smaller so that a greater proportion are beyond the limit of resolution of the instrument. If it is accepted that the distribution should be logarithmic normal, there is no need to estimate a figure for the total weight and the way this is done in programming the computer has been outlined. The calculated line derived from the computer figures is also shown in Fig. 2.

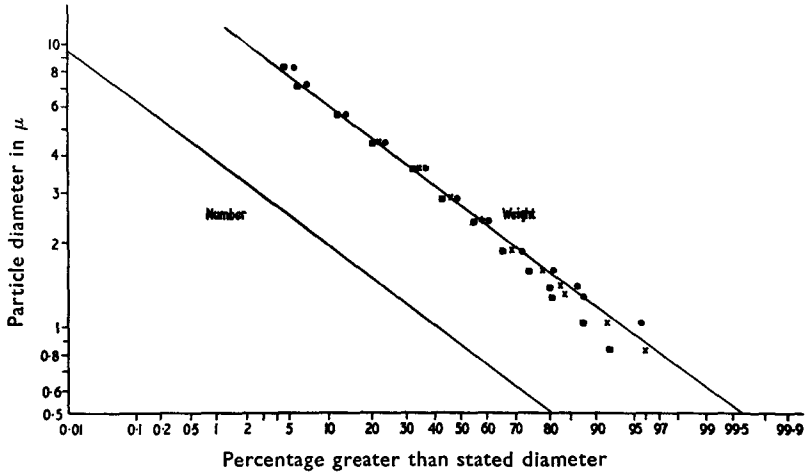

FIG. 2. The experimental points are plotted, assuming three different totals for the volume of *all* particles, including those smaller than the limit of resolution of the Coulter Counter. The weight line is that corresponding to the distribution printed out by the Mercury computer. The number line is derived theoretically from the weight line (see text). ■ $\epsilon = 1{\cdot}40 \times 10^{-7}$ cm.^{3} × $\epsilon = 1{\cdot}35 \times 10^{-7}$ cm.^{3} ● $\epsilon = 1{\cdot}30 \times 10^{-7}$ cm.^{3}

The absolute lower limit of size with the apparatus described is about 0·8 μ. From Table III it can be seen that the number of particles counted in each size range, N, is still very large at this diameter, suggesting that the sample contains many particles too small to be counted. As a theoretical check of this, the logarithmic normal weight curve can be transformed into a frequency, or number, curve which will then give the number average diameter or that diameter below which the number of particles should begin to decrease. The relationship between weight and number of particles is

$$\log d(w) = \log d(n) + 6{\cdot}907 \log^2\alpha$$

where d(w) and d(n) are diameters of particles such that the percentage greater than d, by weight and number, has the same numerical value.

Thus, from Fig. 2, 50 per cent by weight of the particles are greater than 2·85 μ (d $= 2{\cdot}85\mu$) and $\alpha = 1{\cdot}875$. The corresponding value of d(n) from the above equation is 0·87 μ which means that 50 per cent by number are greater than 0·87 μ. This is also the geometric mean diameter, M. From Fig. 2, also, it can be seen that 97 per cent by weight of the particles have diameters greater than 0·87 μ. Since the minimum diameter counted by the instrument is 0·82 μ (see Table II), only about half the theoretical total number of particles in the suspension have been counted, but because these are the larger particles, they represent 97 per cent of the total weight. The data, is, therefore, not sufficiently complete to enable a full distribution by number to be determined experimentally but, by conversion into a weight distribution, which is common practice, assuming the weight of a particle is proportional to the cube of its diameter, the data covers 97 per cent of the distribution.

The calculated surface area figure is 25,690 cm.^{2}/cm.^{3} or 18,900cm.^{2}/g., taking the density of phenothiazine to be 1·36 g./cm.^{3}.

Discussion

The experimental results, which are typical of many that have been obtained, show that the Coulter counter is a useful apparatus for the determination of particle size distribution within a range of diameters between 1 and 15μ. This range can be extended upwards by substituting a larger aperture but extension below 0·8 μ is not a feasible proposition with the present instrument. By using an electronic computer to calculate the results, fitting them, if applicable, to a logarithmic normal curve by the method described, and calculating the surface area of the particles, a considerable saving in time is effected and operator error is largely eliminated.

It is not universally accepted that particles in a powder or suspension produced by a grinding technique should show a logarithmic normal distribution of size. Our experience is, however, that when there are no gross artificial limitations placed on the spread of particle sizes, such as a sieve in the mill discharge or the collection of "fines" in a separate container, the results fit a logarithmic normal curve within the limits of experimental error. Programmed in the way described, the Mercury computer cannot print out the theoretical distribution if the experimental results are not sufficiently close to a logarithmic normal distribution, and if this occurs, there is strong evidence that the assumption of logarithmic normality cannot be made. This has never happened with the many routine samples of phenothiazine suspension sized by the Coulter counter, but when it does, the information obtained is still of value since the cumulative weight percentages are printed out and this alone is a time-saving operation.

The usefulness of the logarithmic normal distribution, apart from the elimination of the effect of particles too small to be counted, is that it allows an exact value of the specific surface area of the solid particles to be calculated, on the assumption that they are spherical and that their surface

is smooth, i.e. there are no cracks or fissures. Air permeability methods of measuring the surface area of powders make similar assumptions regarding the powder surface so that a direct comparison of surface areas of solids in suspension and in the dry state is possible (Table IV).

TABLE IV

COMPARISON OF SPECIFIC SURFACE AREA OF PHENOTHIAZINE

(Data obtained from Coulter counter and Rigden air permeability apparatus)

Surface area in $cm.^2/g.$	
Coulter counter	Rigden apparatus
11,500	12,400
16,000	17,100
19,200	24,000
23,400	24,300
23,500	27,200
29,200	31,100
32,600	38,800
33,500	43,500

This is of value because the direct measurement of surface area of a dry powder is a simple means of characterising it, although it is not so specific as a complete particle size distribution because different distributions can have the same surface area. Such a method cannot be applied to a suspension of particles in a liquid since the powder must be in the dry state, and the filtering and drying of a suspension often leads to the formation of aggregated particles whose size bears little resemblance to that of the original particles, particularly if surface-active agents are present in the suspension. Difficulties also exist in the reverse process of sizing a dry powder by counting the particles after they have been suspended in an aqueous medium, particularly when the powder is hydrophobic, since true dispersion is hard to achieve without subjecting the suspension to a vigorous shearing process which has a grinding effect on the particles.

If then, it is desired to compare the particle size of a drug when formulated into an aqueous suspension with that of the same drug after it has been finely milled in a dry-grinding machine, the use of the Coulter counter to produce a specific surface area figure for the particles in the suspension and a direct air permeability method to give a similar figure on the dry powder has much to commend it. The need for such measurements occurs when the biological effect of a suspension formulation is to be compared with a tablet formulation of the same drug.

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