[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICS, UNIVERSITY OF MICHIGAN]

Macromolecular Weights Determined by Direct Particle Counting. I. The Weight of the Bushy Stunt Virus Particle[1]

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Introduction

The weights of macromolecules and particles of colloidal dimensions can be determined by a variety of methods: diffusion, osmotic pressure, sedimentation velocity, sedimentation equilibrium, X-ray patterns,[2] light scattering,[3] ultramicroscopy[4] and by direct electron microscopy. Each of these methods has advantages and limitations, and each is particularly applicable only over a certain range of sizes. In the application of any of the methods a determination must be made, either directly or indirectly, of one of two sets of quantities: (1) the volumes and densities of the macromolecular particles, or (2) the number of particles in a known volume of solution and the dry weight of the particles in an aliquot of that volume.

In a general way one would expect that the most reliable method would be the one which most directly determines the quantities involved in the definition of molecular weight: the mass in grams per mole of one molecular species. Any notably indirect method is subject to the assumptions involved in the derivation of the working formulas, and to the reliability of the chain of relations existing between the directly observable quantities and the weight of a gram-molecule. Thus, for example, in the sedimentation equilibrium method, what is directly measured is the space gradient of the index of refraction, or the opacity of the solution in the centrifuge cell. But in order to compute the molecular weight of the solute one must know the variation of the refractive index, or opacity, with concentration of solute, the partial specific volume of the solute while undergoing sedimentation, the density of the solution, the angular velocity of the centrifuge, and, additionally, one must sediment for a sufficient length of time to know that he has achieved equilibrium. Further, one must have reason to believe that the solute molecules do not interact, and that no electrical field gradients are set up by the concentration gradient, a condition best satisfied by the use of a dilute solution, but contrary to the *observational* requirement that a fairly concentrated solution be used.

On the other hand, the limitations on the two direct types of determinations are severe. Accurate measurement of particle size by electron microscopy is limited to particles larger than about $M = 10 \times 10^6$. The possible shrinkage effects of severe desiccation in the microscope make for uncertainty, and, of course, the density of the particle must be known. Determinations of M by ultramicroscopy are severely limited by the fact that, as seen in scattered light, all particles look approximately alike. Hence, one must have an external check on the homogeneity of the specimen. Further, the counting of particles in a suspension is difficult and uncertain, and is restricted to those particles whose size and index of refraction are great enough to allow them to be individually counted. The last restriction has effectively prevented the use of ultramicroscopy in accurately determining the weights of protein macromolecules.

The Method of Direct Particle Counting by Electron Microscopy

A direct method of determining weights of macromolecules has been developed which preserves the theoretical simplicity of ultramicroscopy and removes many of its limitations. The number of macromolecules or particles in a known volume of suspension is counted in the electron microscope and the dry weight of the particles in an aliquot of the known volume is measured. The lower limit of size of molecules is that which is barely countable (*ca.* $M = 70{,}000$), while the electron microscope itself furnishes a fairly reliable estimate of the homogeneity of the particles. The fundamental sources of error are four only: the evaluation of the volume of solution whose particles are being counted, the counts themselves, the degree of homogeneity of the material and the determination of the dry weights. These sources of error will be discussed in detail later. The use of the method of direct particle counts is illustrated in this contribution by a determination of the particle weight of the bushy stunt virus. This virus is chosen because it can be readily grown and highly purified; its particles are exquisitely uniform in size, are easily countable in electron micrographs, and the weight of the virus particles has been previously measured in several laboratories. This particle will hereafter be referred to as the BSV particle.

Experimental

The BSV Preparations.—The BSV particles[5] were obtained from *Datura metaloides* that had been infected with the bushy stunt virus, and were purified by differential centrifugation in a manner similar to that described by

(1) This research has been supported in large part by a grant from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) These five methods are reviewed in Cohn and Edsall, "Proteins, Amino Acids and Peptides," Reinhold Publ. Corp., New York, N. Y., 1943.

(3) Reviewed by Oster, *Chem. Rev.*, **43**, 319 (1948).

(4) Burton, "The Physical Properties of Colloidal Solutions," 3rd ed., Green and Co., New York, 1938.

(5) We are indebted to Mr. Russell Steere for furnishing us with ample quantities of plant juice containing the virus particles.

Stanley.[6] The leaves were frozen for several days, chopped while frozen and the cold juice pressed out through cheese cloth. The juice was then clarified at 4500 r.p.m. in an angle-head centrifuge and the clarified liquid filtered through a thin layer of Celite. The filtered juice was allowed to stand at 4° overnight and decanted into the ultracentrifuge tubes for an acceleration to 50,000 g., followed by immediate deceleration. The clarified juice was then spun at 50,000 g. for seventy-five minutes in a 10° Grebmeier rotor and the supernatant liquid discarded. The pellet was resuspended in fresh double distilled water and clarified at 4500 r.p.m. A sufficient aliquot of the resuspended, clarified pellet so obtained, designated P_1, was set aside for analysis.

The remainder of P_1 was carried through another high-speed sedimentation, resuspension and low-speed clarification cycle. The ultracentrifuge supernatant P_{2s}, as well as the second reworked pellet P_2, was now saved. The cycle was repeated until samples of P_1, P_2, P_3, P_4, as well as the corresponding supernatant suspensions, P_{2s}, P_{3s}, P_{4s} were obtained. Subsequent to the supernatant plant juice first discarded only undissolved material was discarded, and all resuspension volumes were precisely determined. The cycling was completed in seventy-two hours, with the temperature of the suspensions kept near 4° in order to minimize bacterial contamination.

Counting the BSV Particles.—The number of BSV particles per ml. of the seven suspensions obtained as above can be counted in the electron microscope if all of them in a known volume of diluted suspension can be surely observed. It has been found that an adaptation of a spraying technique is suitable for this purpose. (The details of the spraying method employed and an estimate of its reliability will be published elsewhere.[6a]) The BSV-particle suspension was diluted to an appropriate concentration and quantitatively mixed[7] with an aqueous suspension of Dow Latex polystyrene particles[8] *containing a known number of latex particles per ml.* The mixed suspension was then sprayed directly upon collodion-coated microscope screens to form small, approximately circular patterns containing both the latex and the BSV particles. The patterns varied in diameter from about 5 to 30μ and the dilutions were so adjusted that the average pattern contained about 50 latex particles and 100 BSV particles. The numbers of both the latex and BSV particles were counted, thus establishing the number of BSV particles per ml. The droplet patterns were shadow-cast and photographed at a magnification of about 4000×. Enough pictures were obtained to provide about 1000 latex particles for comparison with the number of BSV particles in each suspension.

Calibration of the Latex Particles.—There are several possible ways of determining the number of latex particles per ml., but only one way appeared to be immediately available to us. This consisted of the determination of (a) the volume average of the latex spheres, (b) the density of dry latex and (c) the dry weight of the latex particles in a known volume of a suspension to be used as the standard. The first two measurements yield the weight average of the particles, and this combined with the dry weight gives the number of latex spheres per ml. in the standard suspension.

The method of determining the average diameter of the latex particles has been reported.[8] From the value of the average diameter and an auxiliary curve of size dispersion (see below) the average volume of the particles was computed. The density was measured by immersing chips of thoroughly dried material in aqueous copper sulfate solutions of known densities.

The Dry Weight of the BSV Suspensions.—Small amounts (approx. 10 mg.) of the resuspended and clarified pellets of BSV were placed in weighing bottles and dried to constant weight over P_2O_5 *in vacuo*. The usual precautions were taken to avoid absorption of moisture while weighing. The dried material was further treated to six hours at 95° in P_2O_5-dried air in a vacuum-type desiccator. The desiccator was then evacuated to an air pressure of 100 μ while still hot, and allowed to cool while evacuated for thirty hours. The dry weights were then redetermined.

Tests for Homogeneity.—Inasmuch as the total weight of the dried material containing the BSV particles is measured, the effect of any non-volatile impurity, not counted as BSV particles, will be to increase the apparent particle weight. A search for impurities of this nature was made in a direct way (microscopically) and in an indirect way (spectrophotometrically): (1) It was found that the electron micrographs could be used as sensitive indicators of impurities. Spray droplets of fairly concentrated BSV particle suspensions, without the addition of latex, were photographed and carefully examined for what would appear to be material other than the BSV particles. The average micrograph of a droplet contained about 2000 BSV particles and all of any existing non-volatile impurity. Bacterial contamination was quantitatively assessed by examining several hundred droplet patterns. Areas of the microscope screens sufficiently large to contain an estimated 5 million BSV particles were scrutinized directly for bacteria as their images were moved across the fluorescent screen of the microscope. (2) The optical density of the BSV suspensions was measured through wave length 2600 Å., the peak of the absorption band of nucleic acid. Suspensions were diluted to contain approximately equal numbers of BSV particles per ml., and the ultraviolet absorption calculated in terms of BSV particles/ml. and also in mg. dry weight/ml. This measurement, like all others, does not give an absolute measure of purity, but rather an indication of relative purity.

Results

The Standard Polystyrene Latex Suspensions.—The average diameter of the polystyrene particles was taken[8] to be 2590 ± 25 Å. Recalculation with the aid of the distribution curve shown in Fig. 1, results in a diameter of 2600 Å. for the particle of average volume. The dry density of the latex was found to be 1.052 ± 0.001 g./cc., and the dry weight of 4.00 ml. of the standard sus-

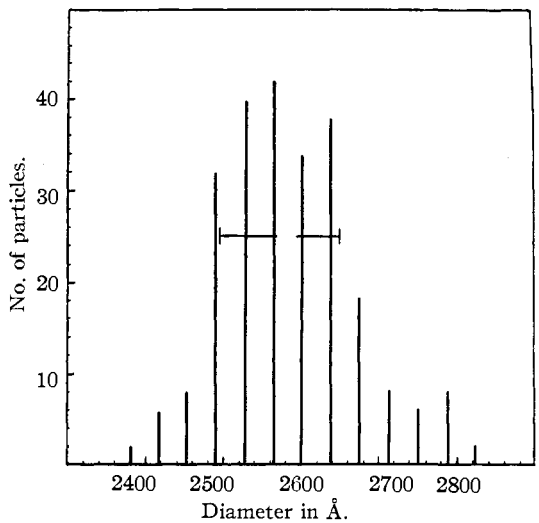

Fig. 1.—Dispersion curve of the diameters of 225 particles of Dow polystyrene latex. Horizontal line shows that 70% of the particles fall within a diameter range of ±3%.

(6) Stanley, *J. Biol. Chem.*, **135**, 437 (1940).

(6a) Scheduled for *J. Applied Phys.*, January, 1950.

(7) Chamot and Mason, "Handbook of Chemical Microscopy," Vol. I, 2nd ed., John Wiley and Sons, Inc., New York, N. Y., 1938, p. 441.

(8) Backus and Williams, *J. Applied Phys.*, **20**, 224 (1949).

TABLE I

DATA FOR THE DETERMINATION OF THE WEIGHT OF THE BSV PARTICLE BY COUNTS

Pellet	Total no. of PSL[a] counted	Ratio[b] of BSV/PSL	Dil. of BSV as sprayed[c]	No. of BSV/ml. in spray ($\times 10^{-10}$)	No. of BSV/ml. as weighed ($\times 10^{-14}$)	Dry wt. of BSV susp. (mg./ml.[d])	Wt. of one BSV particle (gm. $\times 10^{17}$)	Wt. of one BSV particle (mol. wt. units $\times 10^{-6}$)
P_1	970	1.74	8000	7.76	6.21	10.8	1.74	10.4
P_2	1032	1.56	6000	6.96	4.18	7.30	1.74	10.4
P_3	1175	1.66	8000	7.41	5.93	9.00	1.52	9.2
P_4	1095	2.72	2000	12.1	2.42	3.90	1.61	9.6

[a] PSL = polystyrene latex particles. [b] Ratio of numbers of BSV particles to PSL particles as sprayed. [c] Dilution compared to the suspension as weighed. [d] Total weights were from 7.40 to 15.2 mg. No measurable difference between heated dried and unheated dried samples.

pension was found to be 69.2 ± 0.05 mg. The standard suspension was diluted 40-fold for spraying, and the dilution for each set of BSV particle counts was separately pipetted. Simple calculations show that the number of latex particles per ml. as sprayed was 4.46×10^{10}.

The Counts, Weights, and Particle Weights of BSV Particles.—In Table I are shown the results of the counts, dry weights, and particle weights obtained from the preparations P_1, P_2, P_3 and P_4.

Ultraviolet Absorption.—The data and results of the measurement of optical density at 2600 Å. are shown in Table II.

TABLE II

ULTRAVIOLET ABSORPTION OF BSV PREPARATIONS

Pellet or supn.	No. of BSV/ml.[a] ($\times 10^{-13}$)	Opt. dens.[b] (D) at 2600 Å.	(D) per BSV/ml. ($\times 10^{-13}$)	Dry wt.,[c] (mg./ml.)	(D) for a 0.1% susp.
P_1	1.03	0.87	0.85	0.018	4.8
P_2	1.29	1.01	.78	.023	4.4
P_{2s}	1.29	1.37	1.06		
P_3	1.17	0.97	0.83	.018	5.4
P_{3s}	1.28	1.05	.82		
P_4	1.28	1.09	.85	.021	5.2
P_{4s}	1.28	1.17	.91		

[a] Calculated from counts and known dilutions. [b] For an absorption cell 1 cm. thick. [c] Dry weight per ml. for a suspension of concentration shown in col. 2. Data available only for pellets.

TABLE III

INTERNAL CHECK ON BSV PARTICLE COUNTS AND DILUTIONS

Pell. or supn. fluid	Vol. in ml. which resus.[a]	Vol. in ml. in subseq. pelleting	No. of BSV/ml. ($\times 10^{-14}$)	No. of BSV in volumes indic. in: Col. 2 ($\times 10^{-13}$)	No. of BSV in volumes indic. in: Col. 3 ($\times 10^{-13}$)	Appar. loss of BSV[b] in: (%)	Appar. loss of BSV[b] in: (mg.)
P_1		8.8	6.21		5.47	9	7.8
P_{2s}	14.4		0.55	0.79			
P_2	10.0	8.0	4.18	4.18	3.34	5	1.7
P_{3s}	14.4		0.52	0.75			
P_3	4.10	2.5	5.87	2.41	1.47	5	1.4
P_{4s}	14.4		0.29	0.42			
P_4	4.00		2.42	0.97			

[a] Volume to which pellet was resuspended; supernatant volume left unchanged. [b] Difference in % between numbers of BSV particles in P_n(col. 6) and $[P_{n+1} + P_{(n+1)s}]$ (col. 5).

Internal Check on BSV Particle Counts and Dilution Factors.—The data provide a convenient internal check on the precision of the counts and of the dilutions. Inasmuch as the solid material in each resuspended and clarified pellet, plus its supernatant, derives from the solid material in the preceding resuspended and clarified pellet (less the known amount removed for analysis), the accuracy of the counting and dilution can be checked by simple subtraction. Thus, for example, P_1 was resuspended in 10 ml. of distilled water and 1.2 ml. removed for assay. The remaining 8.8 ml. were used in the formation of P_2 and P_{2s}. The total number of BSV particles found in the resuspended and clarified P_2 plus P_{2s} should equal that found in the 8.8 ml. of P_1. Table III gives the data and the results of the internal checks.

Discussion

Precision of the Particle Weight Determination.—The evaluation of the number of latex particles per unit volume depends upon two kinds of measurements: the average volume of the latex spheres, and the density of the dried latex. Calculation of average volume is based upon a determination of average diameter and shape discussed elsewhere.[8] Other determinations of average diameter have been privately communicated to us from six different laboratories, and the extremes of average diameter have been 2560 ± 30 and 2610 ± 30 Å. Since the spread in seven independent measurements, by wholly independent methods, is about 2%, it is felt that our adopted value of 2590 Å. is probably reliable to 1%. The average volume can be calculated only after a dispersion curve of relative diameters is obtained. Figure 1 shows a distribution of diameters obtained for 225 particles. When the latex material is handled properly in the microscope, so as to avoid undue heating by the electron beam, its uniformity in size is quite satisfactory. Some small error of finite sampling is introduced, however, by the failure of the latices to be of perfectly uniform diameter. Since small errors in diameter are multiplied by a factor of three when translated to error in volume, a generous estimate of the limit of uncertainty of the volume-average of the latex spheres might accordingly be estimated at 5%.

The density of the dried latex can easily be

measured to an accuracy far greater than 1%. The uncertainty here is whether or not the density of an individual particle is the same as that obtained for the dried chips. The latex dilutions were made with a precision far exceeding 1% and the weight of the dried sample was enough (69.2 mg.) to assure a high precision in weighing. It is concluded that the uncertainty in the figure for the number of latex particles per ml. is about the same as the major uncertainty indicated above (5%) in the volume of a single particle. This estimated 5% uncertainty in the average mass of the latex particles is troublesome in that it enters systematically into all of our determinations of particle weights. However, other methods of measuring either the mass of the particles or the number per ml. are available (by sedimentation velocities in media of different densities, and by light-scattering) and any subsequent refinement in these determinations can be applied directly as a correction to the calculated values of weights determined by this method.

Drying and Weighing the Virus-Containing Material.—Although the BSV suspensions have been dried and weighed in an accepted manner there is still the likelihood that some water has been weighed along with the protein. The error in the weight determination alone has been estimated by repeated weighings, and the standard deviation is about 0.5%. But the unanswerable question as to whether the results represent the anhydrous macromolecular weight of the virus is precisely the same question that plagues any determination of molecular weight of proteins, since *all* methods involve at some stage either a determination of density or of dry weight. It can only be said that our results represent the weight of the BSV particle under conditions of drying involving heating to 95° and prolonged exposure *in vacuo* to phosphorus pentoxide.

The Tests for Purity and Homogeneity of the BSV-Containing Material.—As Pirie[9] and others have adequately discussed, there is no single criterion of purity of a protein preparation, nor does the sum of all criteria offer any proof of purity. Indeed, the concept of purity has meaning only as related to the experimental methods used for detecting impurities.

Electron microscopy coupled with our technique of specimen preparation has been relied upon heavily for indications of impurity in our BSV preparations. The electron microscope has formerly been little used with any confidence for this purpose, owing to the difficulty of defining fields representative of the original suspension, but by spraying droplets containing the purified BSV particles, one obtains a representative field in each drop pattern. Droplets of suspensions of all seven pellets and supernatants have been sprayed in sufficiently concentrated form to result in about 2000 BSV particles per drop pattern, and micrographs of these have been carefully examined. In the case of P_1 and to a less extent in P_2, P_{2s} and P_{4s}, there was some very small-sized material present. Pellets P_3 and P_4 had no detectable inhomogeneous material, although an amount as great as 1% of the total would have been readily observable. (A high-quality, uranium-shadowed micrograph will show the presence of objects as small as 30 Å. in mean diameter, or 1/1000 the volume of a BSV particle.) There are, however, three kinds of impurities essentially undetectable by electron microscopy alone: particles too small to be detectable and which do not form observable aggregates upon drying, material of about the same size and shape as the BSV particles, and material of great size but rarely present. Although there is no way to detect the presence of the first impurity by electron microscopy, it is believed that the great dilutions involved in four cycles of centrifugation would have reduced the concentration of the first impurity to a negligible amount. The second type of impurity will cause little error in our determination of particle weight since it will be counted and weighed along with the BSV particles. The third type, typically to be found as bacteria, could cause serious error, since a medium-sized bacterium is equal in weight to about 20,000 BSV particles. No bacterial material, nor any material of comparable size, was found in a search embracing fields containing about 5 million BSV particles, and the conclusion is that the error in weight due to contamination by particles of relatively great size is less than 1%.

The evidence from the light absorption at 2600 Å., combined with the molecular weight determinations, is indicative of relative purity if we assume that non-BSV proteins contain little, if any, nucleic acid. As Tables I and II show, the nucleic acid absorption per BSV particle is approximately constant in all pelleted material, and even in all supernatant liquids except the first one, P_{2s}. The absorption per unit weight concentration is found to increase significantly for the most completely washed pellets, P_3 and P_4, while the calculated particle weights are smallest for these pellets. This relation is best explained by assuming that in the first two pellets there was some non-BSV material present which added to the dry weights but not to the ultraviolet absorption.

It is to be noted that analytical centrifugation and electrophoresis have not been used as criteria of purity of our preparations. The reason for this deliberate omission is that both of these methods are sensitive only to rather special kinds of impurities: those which are present in sufficient concentration and are sufficiently homogeneous (either with respect to sedimentation velocity or in net ampholytic charge-density) to create detectable skewness in a concentration gradient. Gross amounts of impurities of miscellaneous sedimentation and/or electrophoretic velocities can be in the solutions and remain undetectable by these methods.

(9) Pirie, *Biol. Rev., Cambridge Phil. Soc.*, **15**, 377 (1940).

Precision of the Counts.—The error of counting can be statistically analyzed for the size of the most probable error. Computations of standard deviation have been made for all counts and have fallen between 2 and 4%. From statistical theory it is known that the standard deviation in ascertaining the number ratio of 1000 pairs of particles, if mixing is perfect, should be about $1/\sqrt{1000}$, or 3% (*i. e.*, if the counts show this standard deviation, the mixing would be defined as perfect). There is the possibility of a systematic error in the distribution of particles in the drop patterns; some, for example, may become dislodged from the substrate film before shadowing. This possibility has been checked by running a dilution curve of BSV particles *vs.* latex particles over a 5 × 2-fold series of dilutions. These results will be published in detail later, but they can be summarized briefly by saying that the dilution factors calculated from pipetting are checked to within 4% by the counts.

The internal consistency of dilutions and counts, Table III, is a further check against a systematic error. Some loss is to be expected in going from one pellet to the next pellet and supernatant. Some of the loss will be in the clarification sediment, roughly estimated to be 1 mg. in $P_1 \rightarrow P_2 + P_{2s}$, and to be negligible for the later pellets. In resuspending each pellet, however, care was taken not to include any material clearly outside the pellet and clinging to the side of the ultracentrifuge tube. In the $P_1 \rightarrow P_2 + P_{2s}$ cycle an observable quantity was found here and might amount to 2 or 3 mg. Allowance for these obvious losses reduces the per cent. loss in the $P_1 \rightarrow P_2 + P_{2s}$ operation to about 6%. It is felt that since this over-all check on counts and dilutions does not indicate a discrepancy greater than 6% in any of the three cycles, only the statistically predictable small random errors are present. This conclusion is particularly evident when it is noted from the last two paragraphs that both ratios and sums are internally consistent.

Summary of Estimated Errors and Results.—The errors detailed above, if added unfavorably, would result in an upper limit of error of an individual determination of particle weight of about 10%. The question now arises of how best to average the values of the particle weights obtained from the four pellets. An arithmetic mean would decrease the probability of an accidental error strongly affecting the result, but would neglect the evidences for increased purity in the last two pellets. In view of the strength of this evidence, *the most probable value of the dry weight of the bushy stunt virus particle obtained by the method of direct particle counts is* $9.4 \pm 0.7 \times 10^6$ *in molecular weight units.*

Conclusions Regarding the Method.—The method of measuring macromolecular weights by direct counting has the primary advantage of simplicity and directness. It serves as a proper check on molecular weights obtained by centrifugation owing to its freedom from the complications and assumptions involved in sedimentation studies. Its largest uncertainty at present, at least in the case of the highly-purifiable BSV particles, is the calibration of the concentration of the latex particles. The counts of the particles can be made to within any predetermined accuracy with sufficient patience and, indeed, if all the counts discussed here had been made on a suspension of only one pellet, the standard deviation of counting would have been reduced to about 1%. The drying, weighing, and assessing of impurities are measurements that are common to all determinations of large molecular weights, although the methods of measurement vary. In particular, sedimentation velocity provides a sensitive and convenient way of detecting and measuring one component of a paucidisperse system. Even here, however, polydispersity or undetected impurities will cause trouble, since they affect the precision of the auxiliary diffusion constant.

A particularly inviting aspect of the method of direct particle counts is that the suspensions can be sprayed and counted at astonishingly low concentrations. All other methods of measuring macromolecular weights become observationally more precise as the concentration of solute is increased, but since side effects, such as aggregation, always enter at high concentrations, it is common practice to extrapolate experimental results to infinite dilution. With the particle count method the solute is best sprayed at concentrations less than 1 mg. per liter or 0.0001%, compared with the usual sedimentation concentration of 0.4%. At a dilution of 1 mg. per liter, each BSV particle is allotted an average solvent volume of 10 μ^3, or 100,000 times the volume of the particle. The droplets lose water by evaporation in their flight from the spray gun to the microscope screen, but their particle content is determined by the concentration in the droplet at the moment it becomes a discrete entity in the spray.

There are three limitations on the method: (1) the ubiquitous problem of purity, already discussed; (2) the lower limit of size of particles which are countable in the electron microscope, estimated at present to be at about $M = 70{,}000$ and (3) the necessity of suspending the particles in a medium which is wholly volatile.

Comparison with Other Determinations of the Weight of the Bushy Stunt Virus Particle.—A review of the determinations of the weight of the BSV particle, up to 1943, has been given by Lauffer,[10] and values obtained by sedimentation equilibrium, sedimentation velocity and diffusion and by X-ray patterns are critically examined. More recently Oster[11] has given a preliminary value of the molecular weight from light-scattering measurements. The four different kinds of deter-

(10) In "Colloid Chemistry," Vol. V, Reinhold Publ. Corp., New York, N. Y., 1944, pp. 801–805.

(11) Oster, *Science*, **103**, 306 (1946).

minations have yielded four rather divergent results for the anhydrous particle weight.

Sedimentation equilibrium	7.6×10^6
Sedimentation velocity and diffusion	10.6×10^6
X-Ray analysis	13.0×10^6
Light scattering	9.0×10^6

It is seen that the method of direct particle counts provides a mean value (9.4×10^6) not outside the limits of previous determinations.

Summary

1. A method of determining the particle weights of macromolecules suspended in a volatile medium is described. The method is called that of "direct particle counts," and consists essentially of counting, on electron micrographs, the numbers of macromolecules per unit volume of solution, and of obtaining the dry weight of an aliquot of the unit volume. Representative specimen fields are obtained by a spray method.

2. Dow latex particles of polystyrene are used as reference particles to determine the volumes of the spray droplets, which also contain the macromolecules.

3. The dry weight of centrifugally purified particles of the bushy stunt virus has been determined, and is found to be $9.4 \pm 0.7 \times 10^6$ in molecular weight units.

4. Discussions of sources of error are given, and it is concluded that the primary source of error is in the determination of the weight concentration of the latex particles in the spray droplets.

5. Evidence bearing on the purity of the bushy stunt virus preparations is discussed, and it is concluded that the particle weights of the material in the two most highly purified pellets are the most reliable.

ANN ARBOR, MICH. RECEIVED JUNE 9, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Configurational Correlation of (*levo*)-Glyceraldehyde with (*dextro*)-Lactic Acid by a New Chemical Method[1]

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Freudenberg[3] converted (*dextro*)-glyceric acid to (*dextro*)-lactic acid by methods which did not sever any of the bonds directly attached to the rotational center. (*levo*)-Glyceric acid was obtained by the oxidation of D-(*dextro*)-glyceraldehyde by Wohl and Schellenberg.[4] These data effect a configurational correlation between D-(*dextro*)-glyceraldehyde and (*levo*)-lactic acid and establish the designation D-(*levo*)-lactic acid. We report herein a confirmation of this correlation by a chemical method similar in principle to that employed in correlating L-(*levo*)-glyceraldehyde with L-(*dextro*)-alanine.[5]

Tetraacetyl-2-methyl-D-glucose diethyl thioacetal (I) was reductively desulfurized to yield tetraacetyl-2-methyl-1-desoxy-D-glucitol (II). Deacetylation of II produced 2-methyl-1-desoxy-D-glucitol (III) which on periodate cleavage with subsequent oxidation yielded an O-methyllactic acid (IV) that was isolated as its chromatographically purified *p*-phenylphenacyl ester[6] of m. p. 74–75° and $[\alpha]^{23}D$ −16.5° (*c* 2, benzene). This derivative was found to be identical with that obtained on the methylation of the *p*-phenylphenacyl ester of L-(*dextro*)-lactic acid (V), thus effecting a configurational correlation between L-glyceraldehyde and L-(*dextro*)-lactic acid. Carbon two in I is originally D_g, wherein the subscript[7] denotes the reference standard glyceraldehyde, but this carbon becomes L_g in the product of periodate cleavage in which the aldehyde group of D-glucose has been reduced to the hydrocarbon stage. These operations effectively produce an interchange of groups on this rotatory center with a consequent reversal of configuration.

Incidental to the above transformations, we describe herein the crystalline tetraacetyl-2-methyl *aldehydo*-D-glucose obtained by demercaptalation of I according to general technics previously described.[8,9]

Experimental[10]

Tetraacetyl-2-methyl-D-glucose Diethyl Thioacetal (I).[11]—2-Methyl-D-glucose diethyl thioacetal[12] (20 g.) was acetylated by overnight treatment at room temperature with acetic anydride (150 ml.) and pyridine (75 ml.). Crystalline material of fair purity was obtained on pouring the reaction mixture into 3 liters of ice and water; yield 27 g. Pure material was obtained on further crystalliza-

(1) This correlation was reported in *Abstracts Papers Am. Chem. Soc.*, **113**, 13Q (1948).

(2) Bristol Laboratories Research Associate (R. U. L.) and Research Fellow (S. M. O.) of The Ohio State University Research Foundation (Project 224).

(3) K. Freudenberg, *Ber.*, **47**, 2027 (1914).

(4) A. Wohl and R. Schellenberg, *Ber.*, **55**, 1404 (1922).

(5) M. L. Wolfrom, R. U. Lemiux and S. M. Olin, THIS JOURNAL, **71**, 2870 (1949).

(6) Following the general procedure of J. G. Kirchner, A. N. Prater and A. J. Haagen-Smit, *Ind. Eng. Chem., Anal. Ed.*, **18**, 31 (1946) recommended for the separation of fatty acids.

(7) E. J. Crane, *Chem. Eng. News*, **25**, 1364 (1947).

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(10) Unless otherwise noted, all experimental work was performed by Mr. S. M. Olin.

(11) Experimental work by D. I. Weisblat.

(12) T. Lieser and E. Leckzyck, *Ann.*, **511**, 137 (1934).