CHROM. 12,185

CRITICAL ASSESSMENT OF PARTICLE SIZE ANALYSIS OF POROUS SILICA MICROBEAD HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC PACKINGS BY PHOTOSEDIMENTATION

K. K. UNGER and M. G. GIMPEL

Institut für Anorganische Chemie und Analytische Chemie, Johannes Gutenberg-Universität, 6500 Mainz (G.F.R.)

SUMMARY

Particle size analysis of commercial spherical silica packings of graduated pore size by means of photosedimentation (PS) is critically examined and compared to results obtained by microscopy. To avoid particle fractionation, the suspension prepared for PS measurements should be subjected to gentle ultrasonic treatment. For computing the particle size distribution, the effective density of solvent-filled porous particles has to be inserted into the Stokes equation. Reproducibility of d_p, estimated by PS is found to be largely dependent upon the accuracy of the specific pore volume determination. Satisfactory agreement of data between PS and microscopy is obtained for all products studied except for that containing large macropores.

INTRODUCTION

Modern high-performance liquid chromatographic (HPLC) columns are packed with totally porous microparticles whose mean particle diameters, d_p, range between 5 and 10 μm. Usually, the nominal value of d_p is designated on commercial batches of packings. Some suppliers also give representative data on the size distribution such as the $d_{p_{10}}$, $d_{p_{50}}$ and $d_{p_{90}}$ value or the standard deviation, σ, of the mean, respectively. For well-sized materials, σ usually amounts to $\pm$ 10–20%.

It is well-known in the size analysis of powders[1] that several d_p values can be derived depending on the method used and its underlying assumptions. The most common methods applied to particles in the 1–20 μm size range are listed in Table I. Furthermore, the presentation of d_p can be affected by various weighting factors such as number, weight, volume and surface area average which necessarily do not coincide[2]. Finally, a given distribution can be chracterized by a series of averages such an arithmetic mean, median, mode, etc. From these considerations it is obvious that d_p must be accompanied by additional information for effective discussions about the mean particle size of a packing in relation to the efficiency of columns.

The question as to which method is best suited to size analysis of HPLC packings with regard to reproducible, accurate and rapid measurements is difficult

TABLE I

METHODS OF SIZE DETERMINATION OF PARTICLES IN THE 1 TO 20 μm SIZE RANGE

Method	Definition of particle size	Designation
Microscopy	Projected area diameter, which corresponds to the diameter of a circle having the same area as the projected area of the particle resting in a stable position	d_p^a
Sedimentation	Stokes diameter, which corresponds to the free-falling diameter of a particle in the laminar flow region ($Re < 0.2$)	d_p^{St}
Electrical sensing zone method (coulter counter)	Volume diameter which corresponds to the diameter of a sphere having the same volume as the particle (exactly, d_p^c corresponds to number volume diameter)	d_p^c
Wet sieving, applying precision sieves and ultrasonic treatment	Sieve diameter which corresponds to the width of the minimum square aperture through which the particle will pass	d_p^A

to answer. In wet sieving, considerable progress has been made but the procedure is still very laborious and time-consuming. Microscopy has the advantage of direct visualization of particles and hence is less liable to be subject to errors in measurement. The analysis time can be reduced by applying modern imaging devices and automatic sizing aids. The electrical zone-sensing method is rapid but requires standardization with reference materials and creates some difficulties in the end-point determination. Another favourable technique frequently applied in size analysis is photosedimentation (PS). Including sample preparation, analysis can be completed within 30 min. In addition, the measurement conditions are comparable in some respects to the settling of material in slurry packing of the column. As suggested by Endele et al.[3], a mean effective particle diameter of the packing can be derived from the column permeability.

This work deals with the size analysis of some selected porous HPLC packings by means of the PS technique. The limits, reproducibility and accuracy of the method are critically discussed. The data obtained are compared with those derived by microscopy.

EXPERIMENTAL

Packings

Table II lists the characteristics of the four microparticulate silica packings employed in this study. The specific pore volume was measured by mercury porosimetry (Model 200; Carlo Erba, Milan, Italy). The pressure range was 1–2000 bar. The pore size nominated corresponds to the mean pore diameter at 50% of the cumulative pore volume distribution. The apparent density due to helium, $d_{app(He)}$ was determined by means of an air pycnometer (Beckman Model 930; Beckman, München, G.F.R.).

Photosedimentation

A known mass of material (*ca.* 0.200 g) was weighed into a glass cylinder (50 × 50 × 200 mm) containing 400 ml of deionized water. The resulting suspension

TABLE II

CHARACTERISTICS OF SILICA PACKINGS

All the packings were commercial products of E. Merck, Darmstadt, G.F.R.

Designation	Specific pore volume, V_p (ml/g)	Mean pore diameter, D (nm)	Apparent particle density due to helium, $d_{app(He)}$ (kg/m³)	Calculated effective density of particles when pores completely filled with liquid water, d_s (kg/m³)
LiChrospher Si 100, $d_p = 10 \mu m$	1.25	10	2.300	1.340
LiChrospher Si 500, $d_p = 10 \mu m$	1.02	50	2.460	1.420
LiChrospher Si 1000, $d_p = 10 \mu m$	1.08	100	2.490	1.400
LiChrospher Si 4000, $d_p = 10 \mu m$	1.14	400	2.490	1.390

was gently agitated by hand for 1 min followed by ultrasonic treatment for 1 min at 20 J/sec (Type 281/101 ultrasonic generator fitted with a Type 250/101 vibrator; KLN, Heppenheim, G.F.R.). The end-point of the treatment was taken as $t = 0$. The sedimentation tank was placed into the photosedimentometer ($t = 0.5$ min), the optical density was measured during 10 min and then the tank was scanned at a constant velocity of 1 cm/min. The photosedimentometer used was home-made as described in detail by Allen[4]. The tank is positioned on a bank which can be moved downwards or upwards with a constant velocity (Type RSU 83/6 NK motor; Berger, Lahr, G.F.R.). The two photocells (Type BPY; Siemens, Karlsruhe, G.F.R.) are positioned oppositely each other. One beam from the light source passes through the sedimentation tank into the measuring cell where it is attenuated by the suspension. The other (reference) beam falls on the reference photocell. The electrical output of both cells is connected to an amplifier (Type TAA 682, Siemens). A pen-recorder registers the change in optical density when the tank is moved down (see Fig. 1 for typical trace).

Computation

The recorder measures the falling time and the falling depth, respectively, as a function of the optical density, D. The Stokes diameter is given by the relationship

$$d_p{}^{St} = \left(\frac{18 \cdot \eta \cdot h}{(d_s - d_f) \cdot g \cdot t} \right)^{1/2} \tag{1}$$

where η is the dynamic viscosity of the liquid, d_f its density, h the falling height, t the falling time, g the gravitational constant and d_s the effective density of the solid.

Since the change in optical density is proportional to the effective cross-sectional area of the particles passing the beam, an average surface area distribution is obtained which is converted into a weight average distribution. A typical set of data is given in Table III, results being taken from Fig. 1.
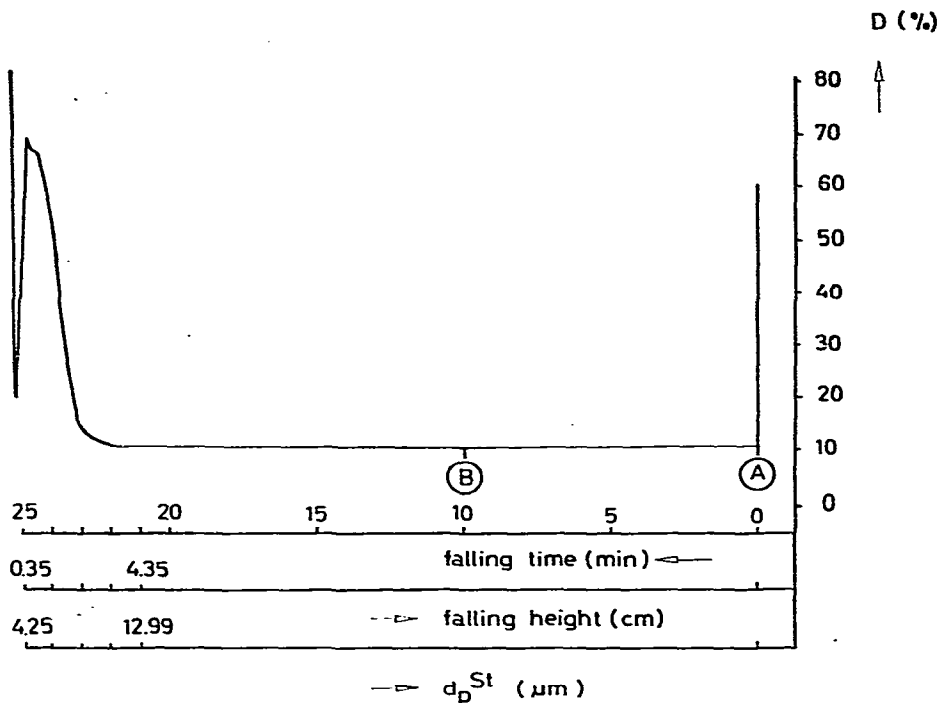
Fig. 1. Pen-recorder trace of a PS analysis (optical density *vs.* time and falling height, respectively) on LiChrospher Si 100, $d_{p_{50}}^{St} = 8.72\,\mu$m. A, Start of measurement; B, start of scanning down the tank at 1 cm/min.

TABLE III

PARTICLE SIZE ANALYSIS OF LiChrospher Si 100, $d_p = 10\,\mu$m BY PHOTOSEDIMENTA-TION

Conditions: $d_s = 1.34 \cdot 10^3$ kg/m³; $d_f = 1 \cdot 10^3$ kg/m³; 293°K; $\eta = 9.4 \cdot 10^{-4}$ Ns/m², sample weight 0.200 g.

Falling time (min)	Optical density, D	d_p^{St} (μm)	Cumulative undersize by surface (%)	ΔD	Average diameter (μm)	Cumulative undersize by weight (%)
21.00	0.9379	14.44	100	0.0122	13.41	100
21.00	0.9256	12.38	98.7	0.0197	11.84	97.7
22.50	0.9060	11.29	96.6	0.0773	10.72	94.5
23.00	0.8287	10.14	88.4	0.0458	9.84	83.0
23.23	0.7830	9.54	83.5	0.1663	9.23	76.8
23.50	0.6166	8.90	65.8	0.1347	8.64	55.5
23.70	0.4819	8.37	51.4	0.1663	7.95	39.4
24.00	0.3151	7.53	33.7	0.0808	7.22	21.1
24.20	0.2348	6.92	25.0	0.0681	6.41	13.0
24.50	0.1666	5.91	17.8	0.0205	5.73	6.9
24.60	0.1461	5.54	15.6	0.0132	5.13	5.3
24.80	0.1330	4.73	14.2	0.1330	2.36	4.4

RESULTS AND DISCUSSION

Errors and limitations of the PS technique

Photosedimentation utilizes gravitational settling of dispersed particles which do not interact, and photoelectric measurement. Under constant conditions at any time t the attenuation of the light beam is proportional to the projected area of particles smaller than d_p^{St}, where d_p^{St} is given by the Stokes equation. This equation is valid for a sphere falling at constant settling velocity in a laminar flow (Reynolds number, $Re < 0.2$). The general limits of the methods are discussed by Allen[5] and are only mentioned briefly here.

The upper limit of particle size that can be measured is given by the dimensions of the photosedimentation device and the operating conditions. Of these the most decisive parameter is the time interval necessary to bring the suspension after dispersion treatment into the measuring position. Assuming $t = 0.5$ min, the maximum particle size d_p^{St} is calculated to be 84 μm for $d_s = 2.3 \cdot 10^3$ kg/m^3 (silica), $d_f = 1.0 \cdot 10^3$ kg/m^3 (water), $\eta = 9 \cdot 10^{-4}$ kg/m$\cdot$sec (water) and $h = 0.15$ m. Because HPLC packings often contain fines with very small particle size, it is more interesting to consider the lower limits of the determination. The following main effects can be distinguished.

(i) The extinction coefficient K_r, defined as "the ratio of light obscured by particles of size d by the light which would be obscured by this particle if the laws of geometric optics held"[5], is only constant when the wavelength of the incident light usually is much less than the particle size. However, for particles smaller than 5 μm, K_r becomes strongly dependent on d_p. In order to account for this dependence the results must be corrected for such diffraction effects by means of a master curve[6,7].

(ii) Brownian motion becomes noticeable for very small particles and may lead to erroneous results, as demonstrated by the following example[8]. The mean distance, at which solid particles ($d_s = 2 \cdot 10^3$ kg/m^3) of $d_p = 1.0$ μm (A) and $d_p = 2.5$ μm (B), respectively, migrate in 1 sec at 294 °K in liquid water amounts to: (A) 0.554 μm due to sedimentation, 0.745 μm due to Brownian motion; (B) 13.84 μm due to sedimentation, 0.334 μm due to Brownian motion.

(iii) The flow pattern of small particles may be affected by convection, especially when the settling velocity of the particle is of the same order as the velocity of the convection current. Since this effect increases with the temperature gradient the temperature should be kept constant.

(iv) The lower size limit also depends on the minimum distance between the light beam and the surface of the liquid, which still permits an accurate measurement of the light intensity. By scanning down the sedimentation tank filled with the suspension, the light beam passing the tank eventually approaches the interface between the dispersing liquid and air. At a certain distance from the interface, the incident light is reflected and does not enter the photocell; hence the intensity I gradually decreases. By blank experiments, the minimum distance of the PS equipment used was determined as 4 mm (see also Fig. 1). Inserting $h = 0.004$ m into the Stokes equation with $t = 25$ min, $d_s = 2.0 \cdot 10^3$ kg/m^3 and $d_f = 1.0 \cdot 10^3$ kg/m^3, the minimum d_p^{St} is calculated to be 2.2 μm.

Errors introduced by ultrasonic treatment

Fine particles of $d_p < 20\ \mu m$ have a tendency to aggregate owing to the high ratio of surface to mass. Ultrasonic treatment is therefore the method of choice when preparing a homogeneous suspension containing essentially isolated particles. During the treatment, however, fracture of the particles may occur depending on the duration of treatment and the energy of the ultrasonic vibrator. To study the behaviour of porous particles, suspensions of LiChrospher Si 100, $d_{p_{sw}}^{st} = 8.7\ \mu m$, were treated with a ultrasonic vibrator at two distinct energy levels at 20 and 100 J/sec, respectively, and at various durations between 1 and 24 min.

The distribution of a suspension was measured by means of photosedimentation and compared to that obtained without ultrasonic treatment. In the latter case the suspension was only agitated gently by hand for 1 min. Fig. 2 shows the amount of undersize by weight smaller than $d_p = 6\ \mu m$ as a function of the duration of ultrasonic treatment at 20 J/sec. It is seen that after a period of ca. 3 min the amount of fines with $d_p < 6\ \mu m$ increases linearly with the duration and reaches 70% (w/w) at 24 min (not indicated in Fig. 2). This example shows that extreme caution is required in the ultrasonic treatment of particles. Because a generalization of the results in this study is not possible, the conditions to prevent fractionation and to maintain the original distribution must be determined for each material under investigation. It should be emphasized that prolonged ultrasonic treatment may also raise the temperature of a suspension.

percentage of cumulative undersize
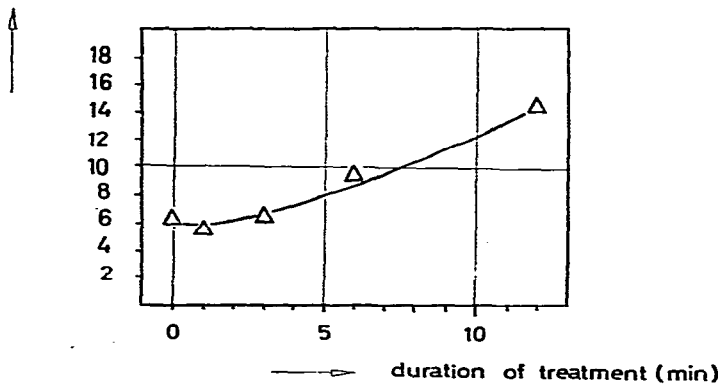by weight smaller than $d_p = 6\ \mu m$ (%)

duration of treatment (min)

Fig. 2. Percentage of cumulative undersize smaller than $d_p < 6\ \mu m$ as a function of the duration of ultrasonic treatment at 20 J/sec before PS measurement on LiChrospher Si 100, $d_{p_{50}}^{st} = 8.72\ \mu m$.

PS measurements of porous particles

By dispersing porous particles for PS measurement the pore volume is completely filled with the sedimentation liquid which is provided to wet the surface. Compared to non-porous particles of equal size, porous partiles will attain a smaller settling velocity, u_{St}, due to lower d_s at sedimentation under comparable conditions, according to the Stokes equation:

$$u_{St} = \frac{(d_s - d_f)\,(d_p^{St})^2 \cdot g}{18 \cdot \eta} \tag{2}$$

In settling porous particles, u_{St} will be a function of the particle porosity. To quantify this effect the effective density, d_s, of liquid-filled porous particles should be calculated and inserted into eqn. 1. d_s is given by

$$d_s = \frac{d_{app(He)} + V_p \cdot d_{app(He)} \cdot d_f}{1 + V_p \cdot d_{app(He)}} \tag{3}$$

where V_p is the specific pore volume. Values of d_s for LiChrospher packings using water as dispersing medium are listed in Table II. It can be easily calculated that u_{St} differs by a factor of six between non-porous and porous particles under constant conditions (d_f, d_p^{St}, η).

For practical applications, particle size analysis by photosedimentation requires the knowledge of both the apparent particle density due to helium *and* the specific pore volume of particles.

As discussed by Svarovsky and Allen[9], the total relative error of d_p^{St}, $\sigma d_p^{St}/d_p^{St}$ depends on the relative errors of of single parameters in the Stokes equation according to

$$\frac{\sigma d_p^{St}}{d_p^{St}} = \frac{1}{2}\left[\left(\frac{\sigma_\eta}{\eta}\right)^2 + \left(\frac{\sigma_\Delta}{\Delta d}\right)^2 + \left(\frac{\sigma_n}{h}\right)^2 + \left(\frac{\sigma_t}{t}\right)^2\right]^{1/2} \tag{4}$$

This means in our case that the precision of the determination of $d_{app(He)}$ and V_p, respectively, will affect directly the reproducibility of d_p derived by PS measurements: for $d_p^{St} = 10\ \mu m$, $d_f = 1 \cdot 10^3$ kg/m³, $\eta = 9 \cdot 10^{-4}$ Ns/m² and $d_s = 2.3 \cdot 10^3$ kg/m³, V_p ranges from 0.0 to 3.0 ml/g. The graphs in Fig. 3 show the dependence of d_p^{St} on V_p, when values are inserted that deviate by $\pm 5\%$, $\pm 10\%$ and $\pm 20\%$ from the true V_p value. In conclusion, to prevent large errors in the estimate of d_p,
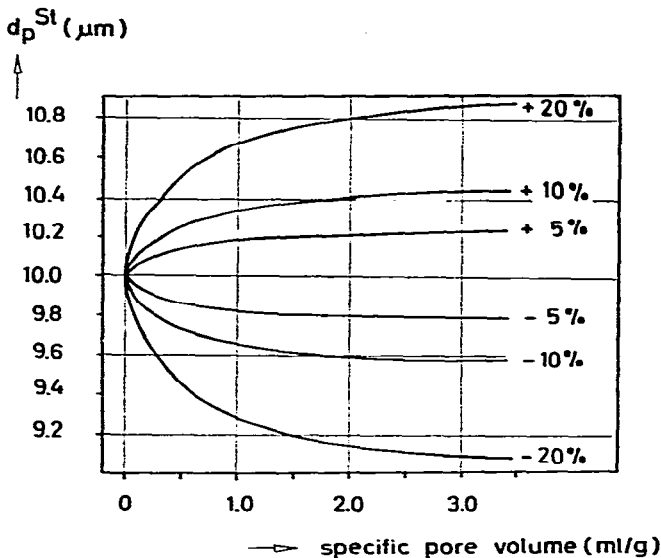

Fig. 3. Variation of Stokes diameter d_p^{St} taken at 10.0 μm with the specific pore volume, V_p, of porous particles under investigation at ± 5, ± 10 and $\pm 20\%$ deviations of V_p from the accurate value.

the determination of V_p should have an accuracy of less than $\pm 10\%$. The reproducibility from several measurements at the same batch of LiChrospher Si 100 was:

$d_{p_{sw}}^{St}$ at 10% of the cumulative undersize distribution by weight $= 6.25 \pm$ 0.26 μm

$d_{p_{sw}}^{St}$ at 50% of the cumulatvie undersize distribution by weight $= 8.31 \pm$ 0.07 μm

$d_{p_{sw}}^{St}$ at 90% of the cumulative undersize distribution by weight $= 10.62 \pm$ 0.21 μm

Comparison of analysis data obtained by PS and microscopy

The spherical silica packings used are ideal model substances to test the agreement of particle size analysis data obtained by different methods. We will only compare the results of photosedimentation and microscopy. Comparative studies of different methods have been made by Perry et al.[10] on glass beads and by Reich[11] on an angular porous silica of $d_p = 10$ μm. Reich found a standard deviation of $\pm 7\%$ for the $d_{p_{50}}$ value averaged from the d_{p50} values from microscopy, photosedimentation, coulter counter and sieving, respectively.

The silica beads under investigation possess nearly the same mean particle size and particle porosity but differ widely in their mean pore diameter (10, 50, 100 and 400 nm, respectively). This gives us the unique opportunity to look for the effect of pore size on the accuracy of size analysis data obtained by PS compared to microscopy. Generally, one expects that at constant particle size the increase in the mean pore diameter will lead to errors in the PS technique, particularly when the pore openings became very large, e.g., a few hundred nanometres. In the case of LiChrospher Si 4000 the mean pore diameter is a factor of 25 smaller than the mean particle diameter, which may influence specifically the sedimentation behaviour. Fig. 4 shows a scanning electron micrograph of such a particle. Table IV lists the experimental results obtained on all packings. Representative values of the cumulative frequency undersize distribution by weight, measured by PS and microscopic counting, are presented together with the deviation between these values. For LiChrospher Si 100, 500 and 1000 the deviation between the corresponding values lies in a reasonable range up to $\pm 12\%$, but large discrepancies up to 40% are observed for LiChrospher Si 4000. It is noticeable that the deviation for the latter is always positive, i.e., photosedimentation gives high values compared to microscopic counting. This result can be explained by the fact that during sedimentation the flow goes almost through the particle and leads to an increase in the settling velocity, u_{St}, compared to porous particles with smaller pores.

CONCLUSIONS

Photosedimentation is a suitable technique for measuring the particle size distribution of silica packings in the 5–10 μm size range. In computing the size analysis data, accurate results will only be obtained by using the effective density of porous particles filled with the sedimentation liquid. The results are found to be in gratifying agreement with microscopic counting except for particles having a large pore diameter of $D = 400$ nm. Pretreatment of particles by ultrasonic vibration should be as gentle as possible to avoid fractionation and hence formation of fines.
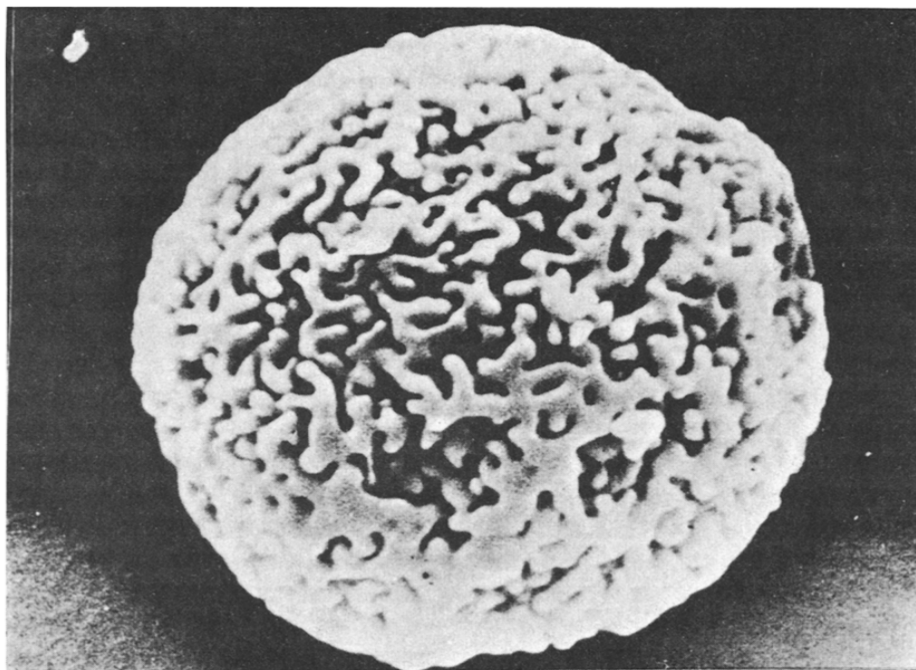
Fig. 4. Scanning electron micrograph of a LiChrospher Si 4000 particle (magnification × 8000).

TABLE IV

COMPARISON OF d_{p10}, d_{p50} AND d_{p90} VALUES

The d_p values were taken from the cumulative frequency undersize distribution by weight by photosedimentation (PS) and microscopy (M)* for silica beads of different mean pore diameter.

Designation of product	Mean pore diameter (nm)	d_{P10} (μm)		Deviation (%)	d_{P50} (μm)		Deviation (%)	d_{P90} (μm)		Deviation (%)
		M	PS		M	PS		M	PS	
LiChrospher Si 100	10	7.4	6.5	−12	9.8	8.6	−12	11.9	11.0	− 8
LiChrospher Si 500	50	7.5	7.5	± 0	10.2	9.4	− 8	13.0	11.8	− 9
LiChrospher Si 1000	100	7.3	7.2	+ 1	10.4	9.9	− 5	14.3	12.6	−12
LiChrospher Si 4000	400	7.4	8.2	+11	9.2	11.0	+20	11.3	16.0	+42

* The authors thank Dr. W. Reich of E. Merck, Darmstadt, for measuring the particle size distribution by microscopy.

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